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Studies on seed colouring in castor, sunflower and safflower

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Abstract

The investigations on the effect of seed colouring on castor (*Ricinus communis* L.), sunflower (*Helianthus annuus* L.) and safflower (*Carthamus tinctorius* L.) seeds encompassing 25 dyes were conducted to identify non-deleterious and deleterious dyes based on their effect on seed quality. The dyes namely Rhodamine-B and Erichro black-T for castor, Rhodamine-B and Cotton blue for sunflower and Rhodamine-B, Fuchsine and Neutral red for safflower are the best dyes for seed colouring at 0.75% concentration. In this paper we discuss effect of seed colouring on seed quality of castor, sunflower and safflower seeds and their resultant implications for Indian seed industry.

Key words: Seed colouring, castor, sunflower, safflower

Introduction

The history of seed colouring in the international arena suggests that colour standards in Canada, United States of America and other European countries were established as per policies regarding colouration of treated seeds and trade memorandums that were issued on July 13, 1967. Different types of dyes have been used for colouring seeds, including acids dyes, basic dyes, direct dyes and pigments. The basic dyes are used most frequently because of their strong, brilliant shades, which can provide distinctive colour in spite of the natural colouration of the seeds, and because of their economy, on an equal colour basis, versus other dye types. The dye is added to the seeds as solution or suspension and blended to give an even coverage. The processors colour seeds because it is required by law to avoid accidental use of treated seeds as food or feed. Some people colour seeds with a specific colour as a trademark, just to identify their seeds. But, to use such dyes, which in fact are chemical formulations, it is necessary to prove that they are non-toxic with respect to seed germination, vigour potential and viability.

There are very few studies in India on seed colouring (Tonapi and Karivaratharaju, 1994) in sorghum, Vivekanandan (1999) and Basavaraj and Kurdikeri (1999) in soybean to establish indigenous colour standards to pave the way for colouring the seeds by incorporating the provisions in the seed quality control and seed trade in India. In this paper we discuss effect of seed colouring on seed quality of castor, sunflower and safflower seeds and their resultant implications for Indian seed industry.

Materials and methods

The investigations on the effect of seed colouring on castor (Cv.PCS-4), sunflower (Cv. Morden and BSH-1), and safflower seeds (Cv. Bheema) encompassing 25 dyes were conducted during, 2001-02 to develop and recommend colour standards after assessing their effect on seed quality.

Dyes used in seed colouring: A total of 25 dyes namely Indigo caramine ($C_{16}H_8N_2O_2$ $(So_3Na)_2$), Titan yellow (Dehydrothio-P-toluidine), Methyl orange($Me_2NC_6H_4N : NC_6H_4SO_2Na$), Methyl Red ($Me_3NC_6H_4N : NC_6H_4 COOH$), Nigrosine ($C_{38}H_{27}N_3$), Erichro Black-T ($C_{20}H_{12}N_3O_7Sna$), Ammonium purpureate ((Mureoxide) $(NH_4)_4$ P_2O_7), Boromocresol green ($C_{21}H_{14}Br_4O_5S$), Bromocresol purple ($C_{19}H_{10}Br_2Cl_2O_2S$), Crystal violet ($C_{25}H_{30}ClN_3$), Malachite green ($Me_2NC_6H_4C_6H_5C : NC_6H_4Me_2Cl$), Congo red ($C_{32}H_{22}O_6N_6S_2Na_2$), Phenol Red ($C_{19}H_{14}O_5S$), Cotton Blue ($C_{32}H_{25}N_3O_9S_3Na_2$), Gentian violet ($C_{25}H_{30}ClN_3$), Fuchsine ($C_{20}H_{17}N_3Na_2O_9S_3$), Methylene Blue ($C_{19}H_{10}Br_2C_{12}O_25$), Rhodamine-B ($C_{28}H_{31}Cl N_2O_3$), Neutral red ($Me_2NC_6H_3N : NC_6H_2MeNH_2.HCl$), Fast green ($C_{37}H_{34}N_2Na_2O_{10}S_3$) along with commercially available dyes in the market namely Kumkum, Yellow, Pink, Blue and Brick red and control (no colouring) were used to develop and recommend color standards after assessing their effect on seed quality.

Preparation of the dye solution: All the dyes were prepared at 0.75% concentration by dissolving 0.25 g of dye in 16.5 ml water and 15.0 ml ethylene glycol (Tonapi, 1988). This concentration was arrived at based on seed colouring trails at the range from 0.25 to 1.5% concentrations.

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Seed colouring procedure: In order to obtain the desired dye intensity, individual dye solutions in specified quantities were added on to 10 g of seeds of each variety placed in a 1000 ml Erlenmayer flask, slowly down the sides of flask with a pipette. The flask was shaken for 3-5 minutes to give uniform coverage of individual dye to the seed. The seeds thus coloured were subjected for laboratory evaluation to assess the effect of these dyes on seed germination, seedling traits, field emergence, electrical conductivity, speed of germination and enzyme activity in seeds to identify the non-deleterious dyes.

Survey to identify best colour: The standardization of 0.75% concentrations was based on attractive colouration imparted on to the seeds. Based on the shine, luster, deep uniform and vibrant colouration imparted on to the seeds of castor, sunflower and safflower, a survey to know the final choice and approval of the group encompassing seed corporations, seed growers, processors and seed traders was conducted with the sample size of one hundred individuals.

Observations: The effect of seed colouring dyes on seed germination, root and shoot length of seedlings, dry weight and seedlings, speed of germination, field emergence and speed of germination was recorded following International Seed Testing Association Standards (Anonymous, 1985). Seed vigour was assessed through vigour index calculated as the product of root length and seed germination and expressed as absolute value (Abdul Baki and Anderson, 1973).

Electrical conductivity of seed leachate (Presley, 1958), α-amylase activity (Simpson and Naylor, 1962) and Dehydrogenase activity (Kittock and Law, 1968) were estimated to assess the effect of dyes on biochemical composition of seeds. All the tests were conducted in four replications consisting 400 seeds. Fisher's method of analysis of variance was applied for data analysis.

Results and discussion

Influence of seed colouring on germinative, physiological and biochemical indices of seeds in castor, sunflower and safflower exhibited both positive and deleterious influences on root-shoot and whole seedling length, dry weight of seedlings, vigour index, speed of germination and electrical conductivity of seed leachate for castor, sunflower and safflower are presented respectively in tables 1, 2 and 3. The influence of dyes on seed germinability in laboratory, field, and in d-manitol soak test, chemical soak test and exhaustion test of castor, sunflower and safflower are presented in figures 1,2 and 3 respectively. The results are in line with the findings of Saraswathi (1994), Tonapi and Karivaratharaju (1994), and Tonapi *et al.* (2004).

The detailed analysis of the data inferred to categorise all the 25 dyes into best, mid range and most deleterious in relation to their effect on seed quality. The dyes namely Rhodamine, Erichro black-T, Nigrosine, Cotton blue, Mureoxide and Neutral red were the best and most favoured dyes in view of their promoting effect on seed quality in castor. The most deleterious dyes were identified in castor as Natural dyes Pink and Kumkum and the others were in mid range. The dyes namely Rhodamine-B, Congo red, Bromocresol purple, Nigrosine, Phenol red and Cotton blue were identified as the best and most favoured dyes in view of their promoting effect on seed quality in sunflower. The most deleterious dyes in sunflower are natural dyes Pink, Yellow and Brick red. Remaining dyes were classified as mid range in terms of the fact that they were not very deleterious but they were certainly declined the total seed quality components significantly when compared to most favoured dyes. The dyes namely Rhodamine-B, Fuchsine and Neutral red are the best and most favoured dyes in view of their promoting effect on seed quality in safflower. The most deleterious dyes in safflower natural dyes Blue and Kumkum which are injurious to the seeds as expressed in seed germination, field emergence and the total deleterious impact on physiological and biochemical parameters involved in this investigation. The remaining dyes were classified as mid range.

However, the promotory effect of some of the dyes may be due to the probable stimulatory effect on enzymes like α-amylase and dehydrogenase activity and their release during seed germination, because of which the faster rate of growth of seedlings becomes evident, as seen in the present study; in the form of higher root length, shoot length, and maximum dry weight of seedlings, including vigour index. The inhibitory toxic effect of Natural dyes Kumkum, Blue, Pink, Yellow, Brick red and Methyl red indicated the entry of the dye, though in very small quantity, into the seed, due to which probably the active chemical ingredient groups of each of these deleterious dyes might have interfered with seed energetics, enzyme release and macromolecule degradation in seed during seed germination to result in the form of maximum number of abnormal seedlings, lower vigour and decreased performance under stress conditions as evaluated through exhaustion test (Tonapi *et al.* 2004).

The most favoured seed dyes out of best dyes, already selected in terms of their influence on seed quality and on the basis of visual colour index and hues imparted, are Rhodamine-B and Erichro black-T in castor, Rhodamine-B and Cotton blue in sunflower, and Rhodamine-B, Fuchsine and Neutral red in safflower.

Table 1 Influence of seed colouring on seed germination (%), seedling growth and vigour index in castor (Cv. PCS – 4)

Name of the dye	Root length (cm)	Shoot length (cm)	Whole seedling length (cm)	Dry weight of seedling (g)	Vigour index (RL x G%)	Speed of germination	EC (µ mhos/cm)
Rhodamine-B	15.0	13.4	28.4	0.67	1365	14.2	329
Cotton Blue	15.5	13.5	29.0	0.64	1316	13.4*	330
Fuchsine	13.1	11.3	24.5*	0.56	934*	11.0*	330
Neutral Red	12.4	12.4	24.8*	0.51	1010*	12.0*	329
Gentian violet	13.3	12.8	26.2	0.36*	905*	12.7*	332*
Methylene Blue	13.6	13.4	27.0	0.43*	762*	9.7*	335*
Crystal violet	15.6	12.8	28.5	0.46*	1278	13.5*	329
Congo red	11.8*	10.5*	22.4*	0.53*	1011*	13.3*	330
Fast green	13.3	11.0	24.3*	0.57*	990*	15.0	330
Bromocresol purple	12.2	11.4	23.6*	0.61	927*	13.7*	330
Phenol Red	12.6	12.3	24.9*	0.73	828*	12.9*	337*
Nigrosine	16.2	11.8	28.0	0.51*	1205*	15.1	330
Erichro black-T	16.8	14.9	31.8	0.52*	1101*	13.3*	337*
Mureoxide (Amm.pur)	9.8*	9.3*	19.1*	0.71	837*	16.2	329
Bromocresol green	12.6	12.7	25.3	0.81	955*	13.2*	330
Malachite green	11.6*	11.1	22.8*	0.85	885*	11.8*	330
Methyl Red	11.7*	11.3	23.1*	0.78	768*	12.6*	339*
Methyl Orange	13.5	11.0	24.5*	0.82	1159*	13.9	338*
Titan yellow	10.3*	11.8	26.8	0.33	1207*	15.6	330
Indigo caramine	15.5	12.8	27.8	0.86	1262	13.1*	330
Natural dye -kumkum	12.9	10.8*	23.8*	0.81	1020*	11.7*	338*
Natural dye – yellow	12.7	10.4*	23.2*	0.72	970*	11.7*	340*
Natural dye – pink	12.7	5.9*	18.6*	0.35*	711*	8.4*	339*
Natural dye – Blue	13.0	10.0*	23.0*	0.68	916*	9.8*	340*
Natural dye –Brickred	13.1	10.2*	23.3*	0.64	838*	11.9*	338*
Control	15.8	13.2	29.0	0.77	1490	15.8	329
SEd±	1.8	1.1	1.8	0.08	109.8	0.9	1.3
CD (P=0.05)	3.6	2.3	3.7	0.18	228.5	1.9	2.5

*Significant at P=0.05

Table 2 Influence of seed colouring on seed germination (%), seedling growth and vigour index in sunflower (Cv. Morden)

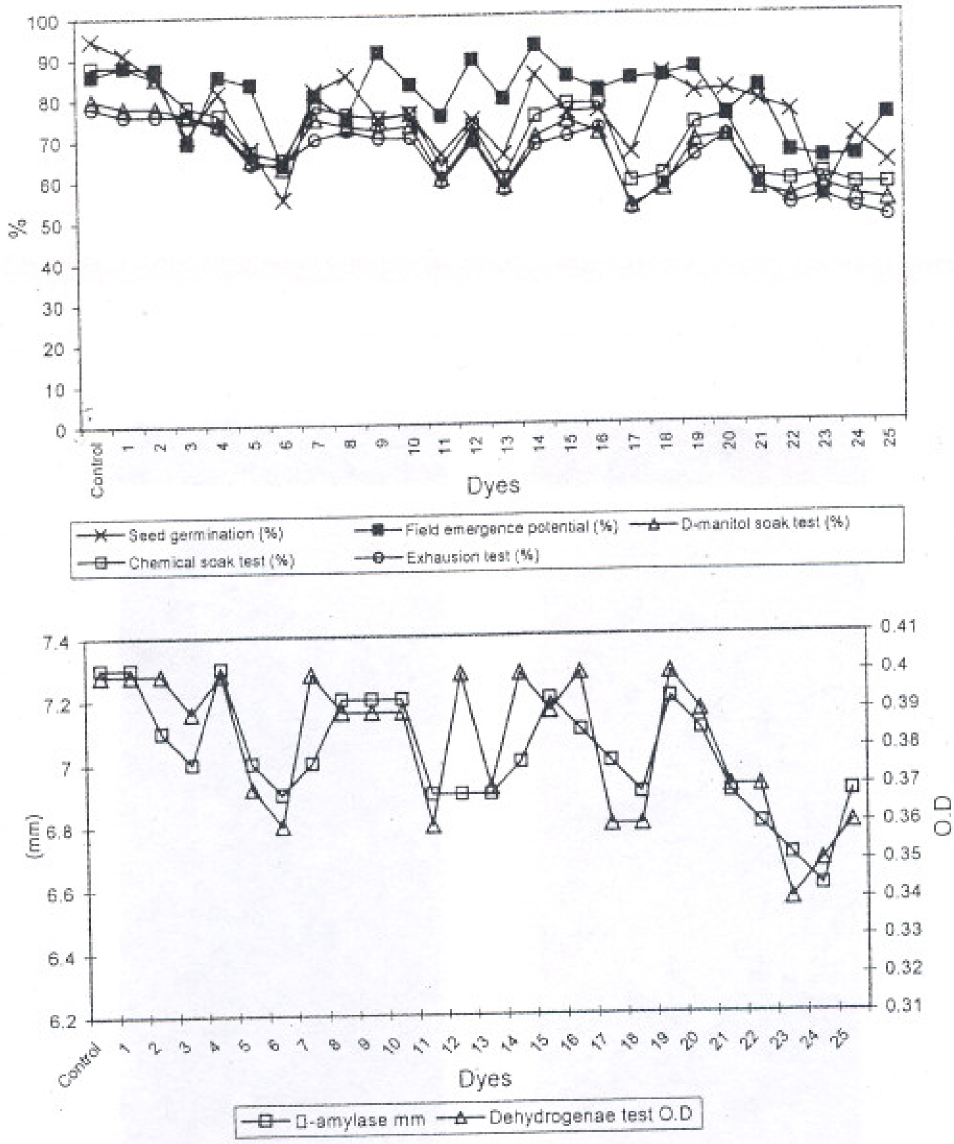
Name of the dye	Root length (cm)	Shoot length (cm)	Whole seedling length (cm)	Dry weight of seedling (g)	Vigour Index (RL x G%)	Speed of germination	EC (μ mhos/cm)
Rhodamine-B	13.4	14.2	27.7	0.13	1180	7.8*	382
Cotton Blue	11.5	14.9	26.4	0.17*	790	10.7	382
Fuchsine	11.6	13.7	25.4	0.17*	893	9.6	383
Neutral Red	12.0	14.8	16.9	0.11	815	6.3*	382
Gentian violet	14.6	14.0	28.8	0.09	904	10.9	381
Methylene Blue	12.3	14.1	26.5	0.11	862	10.7	390*
Crystal violet	14.0	14.4	28.4	0.12	767	14.2	383
Congo red	15.0	15.3	30.4	0.11	1366*	9.8	383
Fast green	13.2	16.5	29.8	0.09	989	11.8	382
Bromocresol purple	15.6*	16.0	31.6*	0.13	1260	10.3	383
Phenol Red	15.5*	16.8*	32.3*	0.11	1068	7.7*	393*
Nigrosine	16.5*	15.2	31.7*	0.14	905	12.3	382
Erichro black-T	12.7	15.1	27.9	0.05	845	10.5	395*
Mureoxide (Amm.pur)	8.2*	13.3	21.5	0.17*	485*	12.0	385
Bromocresol green	13.6	14.3	28.0	0.09	852	13.8	382
Malachite green	11.8	12.9	24.8	0.12	601*	8.0*	383
Methyl Red	11.1	16.3	27.4	0.14	613*	8.1*	393*
Methyl Orange	10.2	15.5	25.8	0.08	536*	12.3	397*
Titan yellow	11.2	13.4	24.6	0.10	954	10.8	382
Indigo caramine	14.1	15.9	30.0	0.13	1050	13.3	383
Natural dye -kumkum	13.8	12.5	26.4	0.13	898	6.9*	397*
Natural dye – yellow	13.3	12.8	26.1	0.10	655	9.9	395*
Natural dye – pink	10.9	11.5*	22.4	0.14	630	0*	395*
Natural dye – Blue	9.0	14.0	23.0	0.11	588*	0*	397*
Natural dye –Brickred	10.1	12.5	22.6	0.12	647	1.8*	398*
Control	11.8	14.2	25.9	0.10	1008	12.2	382
SEd±	1.6	1.2	2.2	0.03	146.2	1.5	2.5
CD (P=0.05)	3.3	2.5	4.5	0.05	304.1	3.1	5.0

*Significant at P=0.05

Table 3 Influence of seed colouring on seed germination (%), seedling growth and vigour index in safflower (Cv. Bheema)

Name of the dye	Root length (cm)	Shoot length (cm)	Whole seedling length (cm)	Dry weight of seedling (g)	Vigour index (RL x G%)	Speed of germination	EC (μ mhos/cm)
Rhodamine-B	10.4	12.9	23.3*	0.16*	989*	16.9*	218
Cotton Blue	11.1	13.8*	25.0	0.12	822*	16.8*	220
Fuchsine	10.4	13.2	23.6*	0.13	1012*	15.2*	219
Neutral Red	12.1	13.5*	25.7	0.11	1152*	21.1	218
Gentian violet	13.3	13.3	26.6	0.09	1169*	17.7*	227*
Methylene Blue	13.1	11.3	24.4*	0.10	1121*	17.8*	229*
Crystal violet	9.3	11.1	20.5*	0.06	892*	19.0	220
Congo red	11.7	13.0	24.8*	0.13	1141*	19.4	220
Fast green	11.1	11.6	22.7*	0.12	1025*	21.2	219
Bromocresol purple	11.7	12.3	24.0*	0.10	1045*	19.1	220
Phenol Red	12.8	12.7	25.5	0.11	956*	20.3	230*
Nigrosine	14.3	13.1	27.4	0.07	934*	19.0	218
Erichro black-T	11.9	12.6	24.5*	0.07	1060*	15.5*	233*
Mureoxide (Amm.pur)	12.5	12.9	25.5	0.09	1164*	13.7*	220
Bromocresol green	11.6	13.8*	25.4	0.08	730*	15.7*	219
Malachite green	14.3	13.3	27.8	0.12	1153*	18.7	218
Methyl Red	10.0	11.0	21.0*	0.08	900*	15.6*	237*
Methyl Orange	11.7	12.3	24.0*	0.15	1059*	17.1*	240*
Titan yellow	11.8	13.1	24.9	0.08	1094*	11.3*	218
Indigo caramine	13.5	14.0*	27.5	0.10	1144*	14.2*	219
Natural dye -kumkum	19.0	10.2*	19.2*	0.14	721*	7.4*	243*
Natural dye – yellow	11.7	12.0	25.8	0.10	1048*	10.3*	244*
Natural dye – pink	10.4	12.0	22.5*	0.10	893*	8.2*	240*
Natural dye – Blue	9.5	11.3	20.8*	0.07	650*	8.2*	241*
Natural dye –Brickred	10.6	12.1	22.7*	0.11	865*	14.2*	245*
Control	15.3	12.1	27.5	0.11	1446	22.4	219
SEd±	3.0	0.6	1.2	0.02	99.2	1.7	1.8
CD (P=0.05)*	6.2	1.3	2.6	0.04	206.4	3.5	3.7

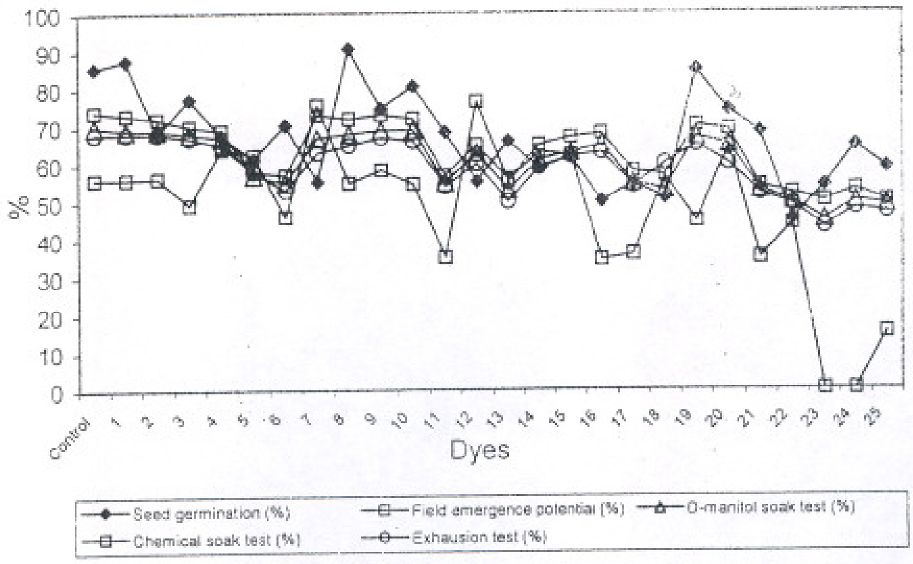
*Significant at P=0.05

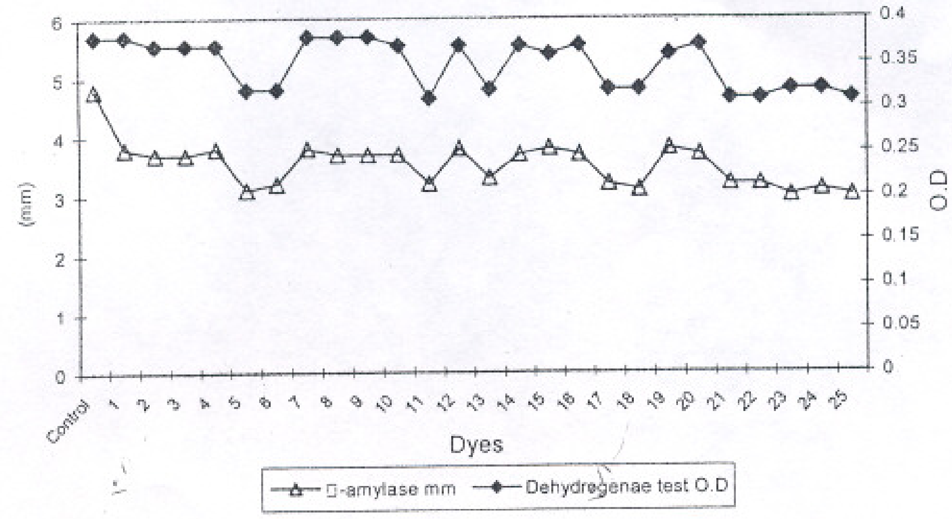


Dyes : Control

1. Rhodamine-B
2. Cotton Blue
3. Fuchsim
4. Neutral Red
5. Gentian violet
6. Methylene Blue
7. Crystal violet
8. Congo red
9. Fast green
10. Bromocresol purple
11. Phenol Red
12. Nigrosine
13. Erichroblack-T
14. Mureoxide (Amm.pur)
15. Bromocresol green
16. Malachite green
17. Methyl Red
18. Methyl Orange
19. Titan yellow
20. Indigocaramine
21. Natural dye-kumkum
22. Natural dye – yellow
23. Natural dye – pink
24. Natural dye – Blue
25. Natural dye –Brickred

Fig.1: Influence of seed colouring on germination potential, field emergence and enzyme activity in castor (PCS - 4)

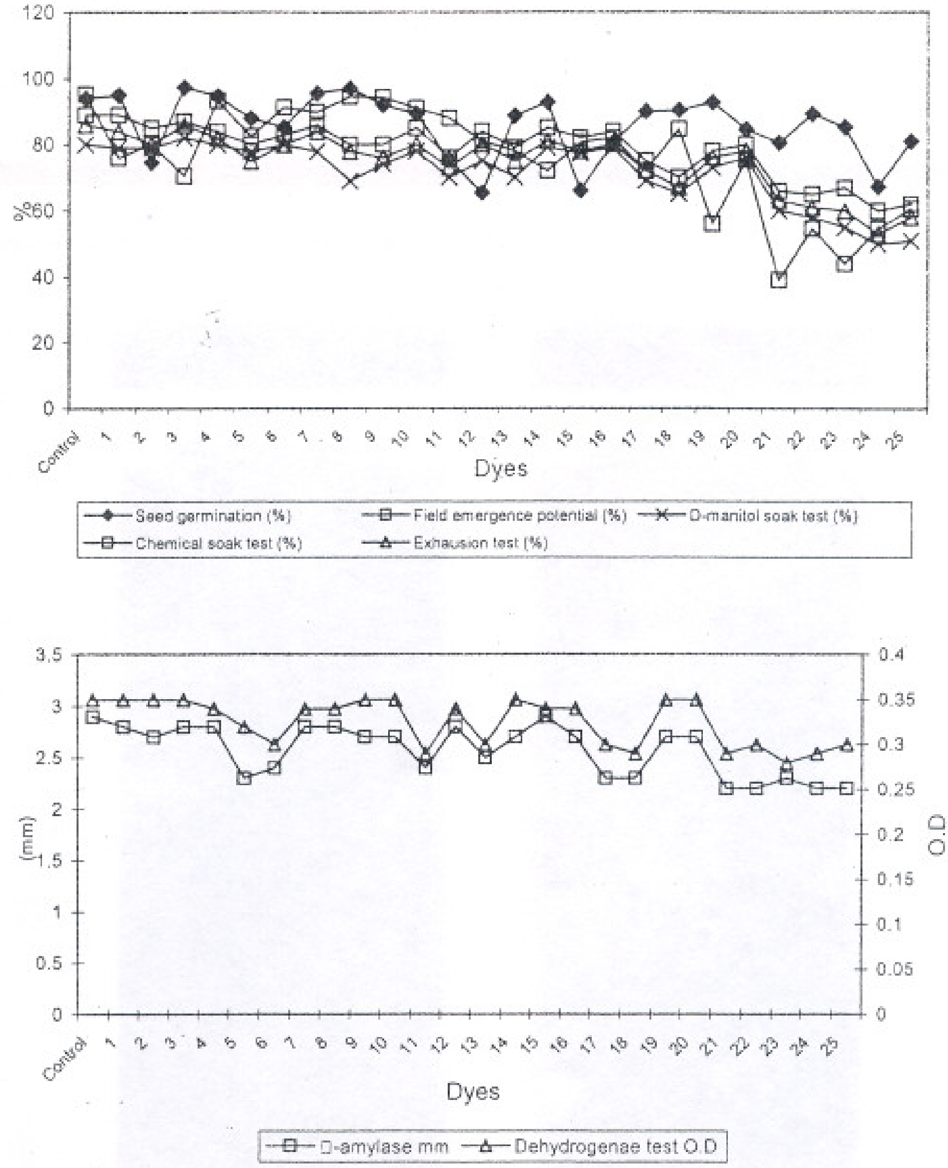




Dyes :

Control
1. Rhodamine-B
2. Cotton Blue
3. Fuchsim
4. Neutral Red
5. Gentian violet
6. Methylene Blue
7. Crystal violet
8. Congo red
9. Fast green
10. Bromocresol purple
11. Phenol Red
12. Nigrosine
13. Erichroblack-T
14. Mureoxide (Amm.pur)
15. Bromocresol green
16. Malachite green
17. Methyl Red
18. Methyl Orange
19. Titan yellow
20. Indigocaramine
21. Natural dye-kumkum
22. Natural dye – yellow
23. Natural dye – pink
24. Natural dye – Blue
25. Natural dye –Brickred

Fig.2 : Influence of seed colouring on germination potential, field emergence and enzyme activity in sunflower (morden)



Dyes : Control

1. Rhodamine-B
2. Cotton Blue
3. Fuchsim
4. Neutral Red
5. Gentian violet
6. Methylene Blue
7. Crystal violet
8. Congo red
9. Fast green
10. Bromocresol purple
11. Phenol Red
12. Nigrosine
13. Erichroblack-T
14. Mureoxide (Amm.pur)
15. Bromocresol green
16. Malachite green
17. Methyl Red
18. Methyl Orange
19. Titan yellow
20. Indigocaramine
21. Natural dye-kumkum
22. Natural dye – yellow
23. Natural dye – pink
24. Natural dye – Blue
25. Natural dye –Brickred

Fig.3 : Influence of seed colouring on germination potential, field emergence and enzyme activity in safflllower (Bheema)

Through this study on colour standards, we would like to propose for efforts to standardize reproducible colour standards for crops as in USA, Canada and Europe. We propose that this provision can also be incorporated under the regulations of seed quality and pest control act after suitable modifications in the text that " Where the physical properties of the control product are such that the presence of the control product may not be recognized when used and is likely to expose a person or domestic animal to severe health risk, the control product shall therefore be denaturized by means of colour, odour or such other means as the central seed committee may approve to provide a signal or warning as to its presence".

When the coloured seed is packed, the package should bear a label with words " the seed is treated and coloured with" followed by the name of the control measure product and the seed colouring dye, including the common name or chemical name of its active ingredient together with appropriate precautionary symbol and signal or warning words as the Central Seed Committee or National Seed Board may approve.

If the treated-coloured seed is sold and shipped or exported in bulk, the shipping documents should bear information containing the common name or chemical name of active ingredient of both chemical and the seed colouring dye with a sub note that "seed colouring dye used is not injurious to seed".

This will enable seed industry to adopt individual colours as their trademark including propriety colouring of parental lines to identify their seeds. The seed colouring will substantially aid in preventing accidental usage of treated seeds as food or feed, or may help in upgrading the visual quality of blonded (discoloured and rain soaked) and blended seeds (but, still maintaining seed germination above seed certification standards under emergent situations where there is scarcity of seeds.

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