Studies on Development of Seed Coloring Standards in Paddy and Maize

VILAS A. TONAPU, P. HARINATH BABU, N. A. ANSARI, S. VARANAVASIAPPAN, RAVINDER REDY Ch., S. S. NAVI AND N. SEETHARAMA

National Research Centre for Sorghum,
Rajendranagar, Hyderabad - 500 030

(Received: August, 2005)

Abstract: The investigations on the effect of seed colouring on paddy and maize 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, Fast green and Fuchsine in order of preference both for paddy and maize are the best dyes for seed colouring at 0.75% concentration. In this paper the possibility of developing colour standards for paddy and maize seeds and their resultant implications for Indian seed industry has been discussed.

Introduction

The history of seed colouring in the international arena suggest 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. In co-operation with CACA Technical Committee, Board of Grain Commissioners and other officials of the Canada department of agriculture, the plant products division has developed a colour standard for cereal seed treatment. This standard was established in order to facilitate treated seed in food or feed grain channels. As of January 1st, 1968 all cereal seed treatment products accepted for registration on renewal under the terms of the pest control products act must confirm to the colour standard. Products appearing on the market as a result of carry over stocks shall not be considered to be in violation of the requirement until January 1st, 1969. In line with this, Canada department of agriculture has given an outline of the laboratory method for the preparation of the standard to which all cereal seed treatment products must be compared when used according to label directions. In addition, in Canada, it is required at the time of submitting application for registration or renewal, a one half pound sample of the cereal seed which has been treated with the candidate product, using the attached procedure at the dosage specified on the product label, must be submitted to the pesticide unit for examination.

Several different types of dyes have been used successfully 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 inspite 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 beverage. However, the quantity of the dye required at 0.75 per cent concentration (prepared by dissolving 0.25 g of dye in 16.50ml water plus 15.0ml of ethylene glycol) varies with the individual dyes used in seed colouring and kind of seed to which the colour is to be imparted. Several dyes have been approved by department of agriculture in Canada and United States of America, viz., Rhodamine-B, Tartrazine, FD and C blue, Methylene blue, Methyl violet 2B, D and C red, D and C violet, D and C green and pigment red, based on their non-toxic nature in regard to seed germinability. To be precise, 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 know that they are not harmful for seed germination, vigour potential and viability, the information about which is not available to the extent it can be used in seed industry. But, till date there are very few and isolated studies in India to establish colour standards to pave the way for coloring the seeds by incorporating the provisions in the seed quality control and seed trade in India. Since the literature scan has indicated absence of any relevant literature in developing colour standards in India except the work of Tonapi and Karivaratharaju (1994) and Tonapi (2004) in cereals, pulses, oil seeds and Tomato, Vivekanandan (1997) and Basavaraj et al. (1999) in soybean. In other countries too, colour standards are propriety of individual private companies. To be precise, processors colour seeds because it is required by law to avoid accidental use of treated seeds as food or feed. Some companies colour seeds with a specific colour as a trademark just to identify their seeds. In this paper we discuss developing colour standards for paddy and maize and their resultant implications for Indian seed industry.

Material and Methods

The investigations, on seed colouring were conducted with Paddy (Cv. MTU 7029, BPT 5204) and Maize (Cv. Saranath, Deccan 101) encompassing 25 dyes namely Rhodamine-B, Cotton blue, Fuchsine, Neutral Red, Gentian Violet. Methylene blue, Crystal violet, Congored, Fast green, Bromocresol Purple, Phenol red, Nigrosine, Eriochrome black T, Ammonium purpureate (mureoxide), Bromocresol green, Malachite green, Methyl red, Methyl orange, Tital yellow, Indigo carmine along with commercially available natural dyes in the market namely Kumkum, Yellow, Pink, Blue and Brick red (Table 1) to develop and recommend color standards after assessing their effect on seed quality. All the dyes were prepared at 0.75% concentration by dissolving 0.25 gm of dye in 16.5 ml water and 15.0 ml ethylene glycol (Tonapi, 1988). 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 Erlenmeyer 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 quality and field emergence as influenced by various biotic and abiotic factors.

The observations on seed germination, root and shoot length of seedlings, dry weight of seedlings, speed of germination, field emergence and seed germination in exhaustion test, speed of germination, was recorded following International Seed Testing Association Standards (Anon., 1996). Seed vigor was assessed through vigor 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), Seed germination in chemical soak test (Vanderlip et al., 1973), Seed germination in D-mannitol soak test (Lad, 1986), a-amylase activity (Simpson and Naylor, 1962) and Dehydrogenase activity (Kittcock and Law, 1968) were evaluated to assess the effect of dyes on biochemical composition of seeds. All the tests were conducted in four replications consisting of 100 seeds each. Fisher's method of analysis of variance was applied for data analysis. Critical differences were calculated at P= 0.05.

Results and Discussion

The seed colouring studies in paddy were conducted on two genotypes BPT 5204 and MTU 7029. Various seed colouring dyes involved in developing colour standards exhibited both
positive and deleterious influences on seed quality parameters viz., seed germination (98.0%), root length of seedlings (12.7 - 0 cm), shoot length of seedlings (8.23 - 0 cm), whole seedling length (20.2 - 0 cm), dry weight of seedlings (0.132 - 0 g), Vigour index (1223 - 0), field emergence potential (92.6 - 0) and speed of germination (21.90 - 0). Both the genotypes were similarly responsive in terms of the above listed seed quality parameters however the dyes had significant impact. The biochemical and enzyme activity including performance under stress had widely ranging influences in terms of membrane integrity as indicated by electrical conductivity (128 - 140 μ mhos/cm), μ-amylase activity (7 - 0.33 mm), dehydrogenase activity (0.47 - 0.26 OD). The range of seed germination as influenced by seed colouring in paddy under stress tests viz., were in the range of (83.66 - 1%), (80.3 - 1%) and (79.6 - 0.66%) in chemical soak test, exhaustion test and D-mannitol soak test respectively (Table 2 and Fig. 1, 2).

The cumulative analysis of the data in case of paddy could identify the best and most deleterious seed colouring dyes based on their influence on seed quality parameters both in terms of promotional and deteriorative influences. Thus proving the fact that all these dyes are either organic or inorganic formulations, and can influence seed quality to a greater extent. The detailed analysis of paddy involving two genotypes

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Dye</th>
<th>Chemical composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Indigo caramine</td>
<td>C₆H₆N₂O₂(SO₄Na)⁴⁻</td>
</tr>
<tr>
<td>2.</td>
<td>Titan yellow</td>
<td>Dehydrothio-P-tolu</td>
</tr>
<tr>
<td>3.</td>
<td>Methyl orange</td>
<td>Me₃NC₆H₄N:N</td>
</tr>
<tr>
<td>4.</td>
<td>Methyl Red</td>
<td>Me₃NC₆H₄N:NC₆H₄COOH</td>
</tr>
<tr>
<td>5.</td>
<td>Nigrosine</td>
<td>C₆H₄N₃</td>
</tr>
<tr>
<td>6.</td>
<td>Enircho Black-T</td>
<td>C₂H₄N₂O₂Sna</td>
</tr>
<tr>
<td>7.</td>
<td>Ammonium purpureate</td>
<td>(Mureoxide)(NH₄)₄PO₄</td>
</tr>
<tr>
<td>8.</td>
<td>Borocresol green</td>
<td>C₆H₄BrO₃S</td>
</tr>
<tr>
<td>9.</td>
<td>Bromocresol purple</td>
<td>C₆H₄BrO₂Cl₂S</td>
</tr>
<tr>
<td>10.</td>
<td>Crystal violet</td>
<td>C₂H₁₀C₁₂N₄</td>
</tr>
<tr>
<td>11.</td>
<td>Malachite green</td>
<td>Me₃NC₆H₄C₂H₄C:</td>
</tr>
<tr>
<td>12.</td>
<td>Congo red</td>
<td>C₆H₂O₃S₄N₄</td>
</tr>
<tr>
<td>13.</td>
<td>Phenol Red</td>
<td>C₆H₄O₃S</td>
</tr>
<tr>
<td>14.</td>
<td>Cotton Blue</td>
<td>C₆H₄NO₃S₄N₄</td>
</tr>
<tr>
<td>15.</td>
<td>Gentian violet</td>
<td>C₂H₁₀C₁₂N₄</td>
</tr>
<tr>
<td>16.</td>
<td>Fuchsin</td>
<td>C₆H₂O₃C₂H₄C:</td>
</tr>
<tr>
<td>17.</td>
<td>Methylene blue</td>
<td>C₆H₈BrC₁₂O₅</td>
</tr>
<tr>
<td>18.</td>
<td>Rhodamine-B</td>
<td>C₆H₁₄Cl₂O₅</td>
</tr>
<tr>
<td>19.</td>
<td>Neutral red</td>
<td>Me₃NC₆H₄N:</td>
</tr>
<tr>
<td>20.</td>
<td>Fast green</td>
<td>NC₆H₄MeNH·HCl</td>
</tr>
<tr>
<td>21.</td>
<td>Natural dye - Kurn kun</td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>Natural dye - Brick Red</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>Natural dye - Blue</td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>Natural dye - Yellow</td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td>Natural dye - Pink</td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

![Fig.1. Influence of seed colouring on germination potential, field emergence and enzyme activity in Paddy Cv.BPT - 5204](image-url)
Table 2. Influence of seed colouring on seed germination (%), seedling growth and vigour index in C. addy (Cv. BPT-5204)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the dye</th>
<th>Seed germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Whole seedling length (cm)</th>
<th>Dry weight of seedling (RL x G%)</th>
<th>Vigour index (RL x G%)</th>
<th>Speed of germination</th>
<th>E.C (μ mhos/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rhodamine-B</td>
<td>87 (69)**</td>
<td>10.21</td>
<td>6.73</td>
<td>16.93</td>
<td>0.081</td>
<td>893</td>
<td>17.13</td>
<td>129</td>
</tr>
<tr>
<td>2.</td>
<td>Cotton Blue</td>
<td>84* (67)</td>
<td>9.93</td>
<td>7.23</td>
<td>17.17</td>
<td>0.086</td>
<td>839</td>
<td>15.80</td>
<td>130</td>
</tr>
<tr>
<td>3.</td>
<td>Fuchsin</td>
<td>97 (84)</td>
<td>10.67</td>
<td>7.76</td>
<td>18.43</td>
<td>0.107</td>
<td>1041*</td>
<td>15.46</td>
<td>129</td>
</tr>
<tr>
<td>4.</td>
<td>Neutral Red</td>
<td>89 (70)</td>
<td>8.88</td>
<td>8.23*</td>
<td>17.10</td>
<td>0.106</td>
<td>790</td>
<td>17.03</td>
<td>129</td>
</tr>
<tr>
<td>5.</td>
<td>Gentian violet</td>
<td>98 (85)</td>
<td>10.63</td>
<td>7.87</td>
<td>18.50</td>
<td>0.093</td>
<td>1046*</td>
<td>15.46</td>
<td>132</td>
</tr>
<tr>
<td>6.</td>
<td>Methylene Blue</td>
<td>97 (84)</td>
<td>9.63</td>
<td>6.33</td>
<td>15.97</td>
<td>0.102</td>
<td>937</td>
<td>14.03*</td>
<td>132</td>
</tr>
<tr>
<td>7.</td>
<td>Crystal violet</td>
<td>90 (75)</td>
<td>9.73</td>
<td>8.20*</td>
<td>17.70</td>
<td>0.087</td>
<td>884</td>
<td>14.80</td>
<td>128</td>
</tr>
<tr>
<td>8.</td>
<td>Congo red</td>
<td>97 (85)</td>
<td>10.27</td>
<td>6.53</td>
<td>16.80</td>
<td>0.103</td>
<td>996</td>
<td>15.56</td>
<td>128</td>
</tr>
<tr>
<td>9.</td>
<td>Fast green</td>
<td>98 (85)</td>
<td>10.90</td>
<td>7.03</td>
<td>18.00</td>
<td>0.103</td>
<td>1069*</td>
<td>14.50*</td>
<td>129</td>
</tr>
<tr>
<td>10.</td>
<td>Bromocresol purple</td>
<td>97 (84)</td>
<td>10.63</td>
<td>7.57</td>
<td>18.20</td>
<td>0.103</td>
<td>1038*</td>
<td>13.93*</td>
<td>130</td>
</tr>
<tr>
<td>11.</td>
<td>Phenol Red</td>
<td>78 (62)</td>
<td>9.37</td>
<td>6.50</td>
<td>15.87</td>
<td>0.092</td>
<td>737</td>
<td>14.56*</td>
<td>135*</td>
</tr>
<tr>
<td>12.</td>
<td>Nigrosine</td>
<td>41* (39)</td>
<td>8.37</td>
<td>4.60*</td>
<td>12.97</td>
<td>0.112</td>
<td>342*</td>
<td>10.73*</td>
<td>130</td>
</tr>
<tr>
<td>13.</td>
<td>Ericho black-T</td>
<td>76 (60)</td>
<td>9.93</td>
<td>6.87</td>
<td>16.80</td>
<td>0.109</td>
<td>759</td>
<td>14.33*</td>
<td>136*</td>
</tr>
<tr>
<td>14.</td>
<td>Mureoxide (Amm.pur)</td>
<td>58 (49)</td>
<td>10.03</td>
<td>6.63</td>
<td>16.67</td>
<td>0.093</td>
<td>582*</td>
<td>4.03*</td>
<td>130</td>
</tr>
<tr>
<td>15.</td>
<td>Bromocresol green</td>
<td>85 (67)</td>
<td>10.10</td>
<td>7.83</td>
<td>17.93</td>
<td>0.092</td>
<td>862</td>
<td>17.26</td>
<td>130</td>
</tr>
<tr>
<td>16.</td>
<td>Malachite green</td>
<td>89 (74)</td>
<td>12.77*</td>
<td>7.43</td>
<td>20.20*</td>
<td>0.094</td>
<td>1140*</td>
<td>13.83*</td>
<td>130</td>
</tr>
<tr>
<td>17.</td>
<td>Methyl Red</td>
<td>10* (15)</td>
<td>5.43*</td>
<td>3.40*</td>
<td>8.83</td>
<td>0.020*</td>
<td>82*</td>
<td>0*</td>
<td>139*</td>
</tr>
<tr>
<td>18.</td>
<td>Methyl Orange</td>
<td>61* (51)</td>
<td>9.37</td>
<td>5.00*</td>
<td>14.37</td>
<td>0.085</td>
<td>577*</td>
<td>9.33*</td>
<td>139*</td>
</tr>
<tr>
<td>19.</td>
<td>Titan yellow</td>
<td>62* (52)</td>
<td>10.93</td>
<td>6.37</td>
<td>17.30</td>
<td>0.088</td>
<td>685*</td>
<td>4.63*</td>
<td>130</td>
</tr>
<tr>
<td>20.</td>
<td>Indigo caramine</td>
<td>94 (78)</td>
<td>10.16</td>
<td>7.24</td>
<td>17.37</td>
<td>0.092</td>
<td>952</td>
<td>8.73*</td>
<td>1306</td>
</tr>
<tr>
<td>21.</td>
<td>Natural dye - kumkum</td>
<td>0* (0)</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.00*</td>
<td>0.0*</td>
<td>3.00*</td>
<td>192*</td>
</tr>
<tr>
<td>22.</td>
<td>Natural dye - yellow</td>
<td>10* (15)</td>
<td>3.63*</td>
<td>1.83*</td>
<td>5.47*</td>
<td>0.013*</td>
<td>54*</td>
<td>0*</td>
<td>195*</td>
</tr>
<tr>
<td>23.</td>
<td>Natural dye - pink</td>
<td>0* (0)</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0*</td>
<td>0*</td>
<td>190*</td>
</tr>
<tr>
<td>24.</td>
<td>Natural dye - Blue</td>
<td>8* (13)</td>
<td>2.32*</td>
<td>1.25*</td>
<td>3.57*</td>
<td>0.013*</td>
<td>29*</td>
<td>0*</td>
<td>183*</td>
</tr>
<tr>
<td>25.</td>
<td>Natural dye - Brickred</td>
<td>16* (23)</td>
<td>8.63</td>
<td>4.77*</td>
<td>13.40</td>
<td>0.027*</td>
<td>140*</td>
<td>10.50*</td>
<td>189*</td>
</tr>
<tr>
<td>26.</td>
<td>Control</td>
<td>95 (79)</td>
<td>9.22</td>
<td>6.83</td>
<td>16.00</td>
<td>0.094</td>
<td>874</td>
<td>16.90</td>
<td>129</td>
</tr>
</tbody>
</table>

S.E.d ± 4.78 1.04 0.63 1.49 0.009 69.97 1.09 1.64
C.D (0.05)*  9.96 2.17 1.31 3.11 0.019 145.54 2.26 3.30

* Significant at P=0.05
**Figures in parentheses are arcsine transformed values.
<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the dye</th>
<th>Seed germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Whole seedling length (cm)</th>
<th>Dry weight of seedling (g)</th>
<th>Vigour index (RL x G%)</th>
<th>Speed of germination (cm)</th>
<th>E.C ($\mu$ mhos/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Rhodamine-B</td>
<td>94 (78)**</td>
<td>12.43</td>
<td>4.70</td>
<td>17.13</td>
<td>0.132</td>
<td>1166*</td>
<td>17.46</td>
<td>128</td>
</tr>
<tr>
<td>2.</td>
<td>Cotton Blue</td>
<td>89 (74)</td>
<td>10.73</td>
<td>4.30*</td>
<td>15.03</td>
<td>0.086</td>
<td>953*</td>
<td>11.16</td>
<td>129</td>
</tr>
<tr>
<td>3.</td>
<td>Fuchsin</td>
<td>84 (67)</td>
<td>11.53</td>
<td>6.08</td>
<td>17.50</td>
<td>0.075</td>
<td>972*</td>
<td>16.70</td>
<td>128</td>
</tr>
<tr>
<td>4.</td>
<td>Neutral Red</td>
<td>94 (78)</td>
<td>10.40</td>
<td>4.90</td>
<td>15.20</td>
<td>0.061*</td>
<td>975*</td>
<td>14.50</td>
<td>128.6</td>
</tr>
<tr>
<td>5.</td>
<td>Gentian violet</td>
<td>90 (75)</td>
<td>11.43</td>
<td>5.67</td>
<td>17.10</td>
<td>0.092</td>
<td>1035*</td>
<td>14.40</td>
<td>135*</td>
</tr>
<tr>
<td>6.</td>
<td>Methylene Blue</td>
<td>98 (85)</td>
<td>10.30</td>
<td>4.50</td>
<td>14.00</td>
<td>0.062</td>
<td>1008*</td>
<td>9.93*</td>
<td>136*</td>
</tr>
<tr>
<td>7.</td>
<td>Crystal violet</td>
<td>85 (67)</td>
<td>11.26</td>
<td>4.37*</td>
<td>15.63</td>
<td>0.085</td>
<td>962*</td>
<td>13.70</td>
<td>128.6</td>
</tr>
<tr>
<td>8.</td>
<td>Congo red</td>
<td>97 (84)</td>
<td>11.97</td>
<td>4.77</td>
<td>16.73</td>
<td>0.105</td>
<td>1168*</td>
<td>10.00</td>
<td>129</td>
</tr>
<tr>
<td>9.</td>
<td>Fast green</td>
<td>80 (63)</td>
<td>12.53</td>
<td>5.23</td>
<td>17.77</td>
<td>0.081</td>
<td>1010*</td>
<td>13.13</td>
<td>128.7</td>
</tr>
<tr>
<td>10.</td>
<td>Bromocresol purple</td>
<td>98 (85)</td>
<td>11.37</td>
<td>6.03</td>
<td>17.40</td>
<td>0.107</td>
<td>1113*</td>
<td>15.03</td>
<td>128</td>
</tr>
<tr>
<td>11.</td>
<td>Phenol Red</td>
<td>84 (66)</td>
<td>10.90</td>
<td>4.97</td>
<td>15.87</td>
<td>0.087</td>
<td>920*</td>
<td>14.33</td>
<td>136*</td>
</tr>
<tr>
<td>12.</td>
<td>Nigrosine</td>
<td>97 (84)</td>
<td>12.53</td>
<td>5.80</td>
<td>18.33</td>
<td>0.063</td>
<td>1223*</td>
<td>20.33</td>
<td>1277</td>
</tr>
<tr>
<td>13.</td>
<td>Erlichio black-T</td>
<td>80 (64)</td>
<td>11.07</td>
<td>4.50</td>
<td>15.57</td>
<td>0.034*</td>
<td>896*</td>
<td>16.13</td>
<td>138*</td>
</tr>
<tr>
<td>14.</td>
<td>Mureoxide (Amm.pur)</td>
<td>15 (22)</td>
<td>9.27</td>
<td>5.40</td>
<td>14.67</td>
<td>0.029*</td>
<td>142*</td>
<td>17.83</td>
<td>128</td>
</tr>
<tr>
<td>15.</td>
<td>Bromocresol green</td>
<td>84 (67)</td>
<td>10.00</td>
<td>4.37*</td>
<td>14.37</td>
<td>0.051*</td>
<td>844*</td>
<td>16.33</td>
<td>129</td>
</tr>
<tr>
<td>16.</td>
<td>Malachite green</td>
<td>90 (72)</td>
<td>12.30</td>
<td>6.73</td>
<td>19.03</td>
<td>0.108</td>
<td>1115*</td>
<td>17.50</td>
<td>129</td>
</tr>
<tr>
<td>17.</td>
<td>Methyl Red</td>
<td>19 (25)</td>
<td>9.80</td>
<td>2.90*</td>
<td>12.70*</td>
<td>0.003*</td>
<td>184*</td>
<td>3.46*</td>
<td>140*</td>
</tr>
<tr>
<td>18.</td>
<td>Methyl Orange</td>
<td>65 (53)</td>
<td>10.53</td>
<td>4.30*</td>
<td>14.83</td>
<td>0.060*</td>
<td>686*</td>
<td>6.56*</td>
<td>140*</td>
</tr>
<tr>
<td>19.</td>
<td>Titan yellow</td>
<td>75 (60)</td>
<td>10.47</td>
<td>3.67*</td>
<td>14.13</td>
<td>0.086</td>
<td>796*</td>
<td>5.36*</td>
<td>128</td>
</tr>
<tr>
<td>20.</td>
<td>Indigo caramine</td>
<td>88 (73)</td>
<td>11.60</td>
<td>5.03</td>
<td>16.63</td>
<td>0.091</td>
<td>1027*</td>
<td>13.1*</td>
<td>129</td>
</tr>
<tr>
<td>21.</td>
<td>Natural dye -kumkum</td>
<td>16 (23)</td>
<td>7.00*</td>
<td>3.07*</td>
<td>10.06*</td>
<td>0.002*</td>
<td>980*</td>
<td>0*</td>
<td>198*</td>
</tr>
<tr>
<td>22.</td>
<td>Natural dye - yellow</td>
<td>22 (27)</td>
<td>6.33*</td>
<td>2.67*</td>
<td>9.00*</td>
<td>0.001*</td>
<td>155*</td>
<td>1.06*</td>
<td>199*</td>
</tr>
<tr>
<td>23.</td>
<td>Natural dye - pink</td>
<td>8 (13)</td>
<td>1.00*</td>
<td>2.03*</td>
<td>2.70*</td>
<td>0.001*</td>
<td>1267*</td>
<td>0*</td>
<td>198*</td>
</tr>
<tr>
<td>24.</td>
<td>Natural dye - Blue</td>
<td>0 (0)</td>
<td>0.00*</td>
<td>0.0*</td>
<td>0.0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>197*</td>
</tr>
<tr>
<td>25.</td>
<td>Natural dye -Brickred</td>
<td>48 (44)</td>
<td>9.00*</td>
<td>4.37*</td>
<td>13.37*</td>
<td>0.019</td>
<td>438*</td>
<td>4.3*</td>
<td>198*</td>
</tr>
<tr>
<td>26.</td>
<td>Control</td>
<td>95 (80)</td>
<td>11.26</td>
<td>5.77</td>
<td>17.03</td>
<td>0.130</td>
<td>743*</td>
<td>21.9</td>
<td>127</td>
</tr>
</tbody>
</table>

S.E.d ** 5.39  1.05  0.63  1.451  0.032  119.21  1.14  1.98

C.D (0.05) * 11.22  2.17  1.32  3.02  0.068  239.38  2.29  3.98

* Significant at P=0.05
**Figures in parentheses are arcsine transformed values.
<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the dye</th>
<th>Seed germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Whole seedling length (cm)</th>
<th>Dry weight of seedling (RL x G%)</th>
<th>Vigour index</th>
<th>Speed of germination</th>
<th>E.C (μ mhos/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rhodamine-B</td>
<td>94 (78)</td>
<td>25.40*</td>
<td>24.60</td>
<td>50.0</td>
<td>1.78</td>
<td>2455*</td>
<td>20.50</td>
<td>169</td>
</tr>
<tr>
<td>2</td>
<td>Cotton Blue</td>
<td>97 (84)</td>
<td>20.50</td>
<td>23.77</td>
<td>44.27</td>
<td>1.78</td>
<td>1988</td>
<td>14.23</td>
<td>168</td>
</tr>
<tr>
<td>3</td>
<td>Fuchsite</td>
<td>94 (79)</td>
<td>22.73</td>
<td>23.40</td>
<td>46.17</td>
<td>1.55</td>
<td>2148</td>
<td>18.66</td>
<td>169</td>
</tr>
<tr>
<td>4</td>
<td>Neutral Red</td>
<td>97 (84)</td>
<td>22.00</td>
<td>24.20</td>
<td>49.53</td>
<td>1.64</td>
<td>2134</td>
<td>17.50</td>
<td>170</td>
</tr>
<tr>
<td>5</td>
<td>Gentian violet</td>
<td>77* (61)</td>
<td>21.83</td>
<td>25.73</td>
<td>47.57</td>
<td>1.30*</td>
<td>1682*</td>
<td>25.20</td>
<td>175*</td>
</tr>
<tr>
<td>6</td>
<td>Methylene Blue</td>
<td>96 (83)</td>
<td>23.53</td>
<td>24.47</td>
<td>48.67</td>
<td>1.43*</td>
<td>2274</td>
<td>24.16</td>
<td>175*</td>
</tr>
<tr>
<td>7</td>
<td>Crystal violet</td>
<td>94 (79)</td>
<td>23.10</td>
<td>28.63*</td>
<td>51.73*</td>
<td>1.52*</td>
<td>2187</td>
<td>24.90</td>
<td>169</td>
</tr>
<tr>
<td>8</td>
<td>Congo red</td>
<td>87 (69)</td>
<td>21.00</td>
<td>28.00</td>
<td>49.00</td>
<td>1.34*</td>
<td>1834</td>
<td>18.60</td>
<td>167</td>
</tr>
<tr>
<td>9</td>
<td>Fast green</td>
<td>94 (79)</td>
<td>21.90</td>
<td>22.26*</td>
<td>49.20</td>
<td>1.20*</td>
<td>2070</td>
<td>27.16</td>
<td>170</td>
</tr>
<tr>
<td>10</td>
<td>Bromocresol purple</td>
<td>93 (78)</td>
<td>19.93</td>
<td>25.20</td>
<td>45.13</td>
<td>1.20*</td>
<td>1866</td>
<td>24.23</td>
<td>170</td>
</tr>
<tr>
<td>11</td>
<td>Phenol Red</td>
<td>85 (67)</td>
<td>22.80</td>
<td>23.47</td>
<td>46.27</td>
<td>1.31*</td>
<td>1951</td>
<td>23.30</td>
<td>174*</td>
</tr>
<tr>
<td>12</td>
<td>Nigrosine</td>
<td>94 (78)</td>
<td>19.33*</td>
<td>27.07</td>
<td>46.40</td>
<td>1.33*</td>
<td>1819</td>
<td>23.03</td>
<td>170</td>
</tr>
<tr>
<td>13</td>
<td>Erichro black-T</td>
<td>84 (67)</td>
<td>20.60</td>
<td>21.80*</td>
<td>42.40*</td>
<td>1.27*</td>
<td>1748</td>
<td>18.26</td>
<td>176*</td>
</tr>
<tr>
<td>14</td>
<td>Murexide (Amm.pur)</td>
<td>80* (64)</td>
<td>18.43*</td>
<td>23.63</td>
<td>42.07*</td>
<td>1.42*</td>
<td>1496*</td>
<td>22.40</td>
<td>169</td>
</tr>
<tr>
<td>15</td>
<td>Bromocresol green</td>
<td>90 (71)</td>
<td>20.80</td>
<td>23.13</td>
<td>43.80*</td>
<td>1.62</td>
<td>1878</td>
<td>19.53</td>
<td>169</td>
</tr>
<tr>
<td>16</td>
<td>Malachite green</td>
<td>94 (78)</td>
<td>22.37</td>
<td>25.47</td>
<td>47.80</td>
<td>1.46*</td>
<td>2109</td>
<td>24.10</td>
<td>169</td>
</tr>
<tr>
<td>17</td>
<td>Methyl Red</td>
<td>85 (67)</td>
<td>21.03</td>
<td>23.30</td>
<td>44.33</td>
<td>1.49*</td>
<td>1788</td>
<td>21.46</td>
<td>176*</td>
</tr>
<tr>
<td>18</td>
<td>Methyl Orange</td>
<td>94 (78)</td>
<td>22.03</td>
<td>24.67</td>
<td>46.70</td>
<td>1.50*</td>
<td>2076</td>
<td>23.96</td>
<td>177*</td>
</tr>
<tr>
<td>19</td>
<td>Titan yellow</td>
<td>76* (60)</td>
<td>22.63</td>
<td>24.20</td>
<td>46.83</td>
<td>1.37*</td>
<td>1726*</td>
<td>22.80</td>
<td>170</td>
</tr>
<tr>
<td>20</td>
<td>Indigo carmine</td>
<td>76* (60)</td>
<td>22.10</td>
<td>27.83</td>
<td>49.93</td>
<td>1.93</td>
<td>1687*</td>
<td>23.43</td>
<td>170</td>
</tr>
<tr>
<td>21</td>
<td>Natural dye -kumkum</td>
<td>21* (27)</td>
<td>10.07*</td>
<td>17.00*</td>
<td>27.07*</td>
<td>1.51*</td>
<td>225*</td>
<td>5.20</td>
<td>179*</td>
</tr>
<tr>
<td>22</td>
<td>Natural dye - yellow</td>
<td>60* (51)</td>
<td>17.93*</td>
<td>21.10*</td>
<td>39.03*</td>
<td>1.25*</td>
<td>1440*</td>
<td>11.70</td>
<td>180*</td>
</tr>
<tr>
<td>23</td>
<td>Natural dye - pink</td>
<td>43* (41)</td>
<td>12.57*</td>
<td>20.43*</td>
<td>33.00*</td>
<td>1.02*</td>
<td>561*</td>
<td>3.20</td>
<td>179*</td>
</tr>
<tr>
<td>24</td>
<td>Natural dye - Blue</td>
<td>75* (60)</td>
<td>18.10*</td>
<td>20.50*</td>
<td>38.60*</td>
<td>1.47*</td>
<td>1365*</td>
<td>8.60</td>
<td>179*</td>
</tr>
<tr>
<td>25</td>
<td>Natural dye - Brickred</td>
<td>83 (70)</td>
<td>17.87*</td>
<td>23.60</td>
<td>41.47*</td>
<td>1.55</td>
<td>1493*</td>
<td>25.43</td>
<td>181*</td>
</tr>
<tr>
<td>26</td>
<td>Control</td>
<td>94 (78)</td>
<td>22.20</td>
<td>25.60</td>
<td>47.80</td>
<td>1.78</td>
<td>2092</td>
<td>19.50</td>
<td>169</td>
</tr>
</tbody>
</table>

S.E.d: 6.51 1.20 1.23 1.74 0.11 167.05 1.53 1.83
C.D (0.05) * 13.54 2.51 2.57 3.63 0.22 347.46 3.18 3.70

* Significant at P=0.05
**Figures in parentheses are arcsine transformed values.
BPT 5204 and MTU 7029 could conveniently help to categorize all the 25 dyes into best, mid range and most deleterious in relation to their effect on seed quality. The dyes namely Neutral red, Fuchsine, Mureoxide, Gentian violet, Congo red, Neutral red, Phenol red and Bromocresol purple, Rhodamine-B, Crystal violet and Fast green as the best and most favoured dyes in view of their promoting effect on seed quality in paddy.

The most deleterious dyes in paddy were Natural dyes Pink, Blue, Yellow and Kumkum since all these natural dyes though they are available in the market are in fact chemical formulation that contain harmful elements 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 in terms of the fact that they were not very deleterious, they were certainly declined the total seed quality components significantly when compared to the most favoured dyes as listed above. Based on vibrant colours, shine hues and uniform colouration imparted to paddy seeds, as compiled from the survey of seed growers, processors and seed industry people out of total a sample of 100, the most preferred dyes in the order of preference are Rhodamine-B, Fast green and Fuchsine.

In maize, the various dyes had both significantly promoting as well as deleterious impact on seed germination (97 - 21%), root length (25.4 - 10.07 cm), shoot length (28.6 - 17.0 cm), whole seedling length (51.7 - 27.07 cm), dry weight of seedlings (1.93 - 1.02 cm), vigour Index (2455 - 225), field emergence (96.0 - 20.0%) and speed of germination (27.1 - 3.2).
The biochemical and enzyme activity including performance under stress had wide ranging influences in terms of membrane integrity as indicated by chemical conductivity (167 - 181 \( \mu \) mhos/cm), a-amylase activity (8.20 - 7.16 mm), dehydrogenase activity (0.40 - 0.28 OD). The range of seed germination as influenced by seed colouring in maize under stress tests viz., were in range 88.0 - 30.3\%, 87.3 - 25.7\%, and 86.0 - 1.67\% in chemical soak test, exhaustion test and D-mannitol soak test respectively (Table 4, Fig. 3).

The detailed analysis of the data in maize could conveniently help 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-B, Crystal violet, Fuchsine and Fast green were categorized as the best and most favoured dyes in view of their promoting effect on seed quality in maize. The most deleterious natural dyes were Kumkum and Pink. Perhaps these dyes may contain harmful elements 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 in terms of the fact that they were not very deleterious, but they certainly declined the total seed quality components significantly when compared to most favoured dyes as listed above. Based on vibrant colours, shine, hues and uniform colouration imparted to maize seeds, as compiled from the survey of seed growers, processors and seed industry people out of total sample of 100, it could be brought that most favoured seed colouring dyes out of the best dyes already enlisted on the basis of visual colour index are Rhodamine-B, Fuchsine and Fast green. The results are in line with the findings of Saraswathi (1994), Tonapi and Karivaratharaju (1994), and Tonapi (2004).

Several different types of dyes have been used successfully 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 inspite 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 beverage. However, the quantity of the dye required at 0.75 per cent concentration (prepared by dissolving 0.25 g of dye in 16.50 ml water plus 15.0 ml of ethylene glycol) varies with the individual dyes used in seed colouring and kind of seed to which the colour is to be imparted. However, the promotory effect of some of the dyes may be due to the probable stimulatory effect on enzymes like \( \mu \)-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.

Hence, efforts to standardize reproducible colour standards for crops as in USA, Canada and Europe are needed. We propose that this provision can also be incorporated under the regulations of seed quality and pest control act after suitable modifications with 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”. Where the seed is packaged, the package should bear a label with words “the seed is treated and colored with” followed by the name of the control measure product and the seed coloring dye, including the common name or chemical name of its active ingredient together with appropriate precautionary symbol and signal or warning words as the seed testing Committee approves. If the treated-colored seed is sold and shipped in bulk, the shipping documents should bear information setting forth the common name or chemical name of active ingredient of both chemical and the seed coloring dye with a sub note that “seed coloring dye used is not injurious to seed”. This will enable seed industry to adopt individual colors as their trademark including propriety coloring of parental lines to identify their seeds and seed coloring may substantially aid in preventing accidental usage of treated seeds as food or feed, or may help in upgrading the visual quality of blended (discolored and rain soaked) and blended seeds (but, still maintaining seed germination at (75%) and above certification standards under emergent situations where there is a scarcity of seeds.

References


SARASWATHI, G., 1994, Studies on seed pelleting in relation to sowing qualities of cotton cv. LRA 5166. M. Sc. (Ag.) thesis Tamil Nadu Agricultural University, Coimbatore, India.


VIVEKANANDAN, R., 1997, New approaches in colouring of crop seeds. M. Sc. (Ag) thesis submitted to Tamil Nadu Agricultural University, Coimbatore, India.