# **Fungicide Seed Treatment and Seed Colour Effects on Seed Vigour and Emergence in Flax**

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## ABSTRACT

In this study, the effect of chemical seed treatment with Captan 0.2% and Carbendazim 0.15% and seed colour on germination, seed vigour and emergence was studied on two populations of flax that were near-isogenic for seed colour. The results showed that in germination test, chemical seed treatment and seed colour had no significant effect on germination. However, in the vigour test, the yellow seeds had significantly lower germination than brown seeds (42.3% *vs.* 73.5%). Chemical seed treatment improved percentage germination in vigour test and Captan was more effective than Carbendazime. Emergence of yellow seeds was significantly lower than that of brown seeds at different temperature conditions and chemical seed treatment improved emergence of flaxseed at temperature conditions of 5°C for seven days followed by temperature of 20°C. The necessity of improving the seed vigour and emergence of yellow-seeded genotypes of flax by breeding programs and/or by chemical seed treatment was confirmed and it seems that chemical seed treatment with Captan is more effective than Carbendazime.

Key Words: Seed treatment; Seed colour; Seed vigour; Emergence; Flax; Captan; Carbendazime

## **INTRODUCTION**

A uniform and good stand establishment is necessary for flaxseed (Linum usitatissimum L.) production since flax does not compete satisfactorily with weeds. Previous studies showed that seed vigour and emergence of flaxseed was negatively affected by yellow seed colour, but it had no significant effect on germination (Saeidi & Rowland, 1999a, b & 2000). Early planting can provide various advantages for crop production, but it may expose the seeds to unfavorable conditions such as low soil temperature, high soil moisture content and microbial activity resulting in a poor seed vigour and stand (Tirvaki & Andrews, 2001). Low temperature is one of the major environmental factors negatively affecting germination (Saeidi & Rowland, 1999a) and seedling emergence (O' Connor & Gusta, 1994) in flax. In comparison to brown flaxseed, lower seed vigour and emergence of yellow seeds has been related to the interaction between seed colour and soil conditions such as low temperature and most likely the soil-microorganisms (Saeidi & Rowland, 1999a & 2000). Reitz et al. (1947) had also found that the lower seed yield of yellow-seeded flax was associated with lower germination and poor stands.

Capability of emergence at stress conditions such as low temperature and soil-microorganisms may be improved by chemical seed treatment. Improvement of seed germination or emergence due to seed treatment with fungicides has been reported in different crops such as cowpea (*Vigna unguiculata* L.) (Van den Berg *et al.*, 2002), longleaf pine (*Pinus palustris* Mill.) (Allen *et al.*, 2004), cotton (Lisker & Meiri, 1992) and flax (Schuster *et al.*, 1943; Kommedahl *et al.*, 1955). The objective of this study was to investigate the effect of different fungicide seed treatment and their interaction with seed colour (brown & yellow) on germination, seed vigour and emergence in flax.

## MATERIALS AND METHODS

The same number of seeds from ten lines of nearisogenic populations (Burton, 1966) for brown and yellow seed colour was composited and used for this study to investigate the effect of seed colour on germination and emergence. Pooling the seeds from a number of homozygous plants in a segregating population from two parental lines provided two near-isogenic populations identical except for germination and emergence (Burton, 1966). The brown and yellow treated seeds with the fungicides like Captan 0.2% (50% WP) and Carbendazim 015% (60% WT) along with non-treated seeds (as control) were used in the experiments.

**Germination test.** A factorial experiment with two factors, including two seeds colour and three chemical seed treatment (Captan, Carbendazim & the control) in a randomized complete block design with four replications was used in germination test. The experimental unit consisted of fifty seeds from each treatment in a Petri dish (90 mm diameter). The seeds were placed on two filter papers and incubated in incubators in constant temperature of 20°C, with an eighteen hours photoperiod. During the experiment, the seeds were moistened once every two or three days with distilled water to keep them continuously damp. After seven days, the germinated seeds (at the

dicotyledon stage) were counted and the percentage germination was calculated for each experimental unit.

Vigour test. The seed vigour was measured by cold test method (AOSA, 1983). In the vigour test, a factorial experiment including three factors of seed colour (brown & vellow), seed treatment (with Captan, Carbendazime & nontreated seeds) and type of soil (autoclaved, non-autoclaved & without soil) were used to evaluated their effects on seed vigour using a randomized complete block design with four replications. The experimental unit consisted of fifty seeds from each treatment in a Petri dish (9 cm diameter) on filter paper. For treating the first two levels of soil factor, the seeds of corresponding experimental units were lightly sprinkled with screened and autoclaved or non-autoclaved soil from the field, thus that the seeds could be still visible. The soil was autoclaved for two hours. Seeds were incubated in an incubator with an eighteen hours photoperiod, for seven days at 5°C following by four days at 20°C (Saeidi & Rowland, 1999a). During the experiment, adequate distilled water was added to Petri dishes to keep the seed continuously damp and at the end of experiment, the percentage of germinated seeds (at the dicotyledon stage) was calculated for each experimental unit as an index of seed vigour.

Emergence experiments. In these experiments, the emergences of the seeds for a factorial combination of seed colour and chemical seed treatment were measured in autoclaved and non-autoclaved soil  $(2 \times 3 \times 2 \text{ factorial})$ experiment) at two different temperature regimes. A randomized complete block design with 5 replications was used in the experiments. The experimental unit consisted of fifty seeds, which were planted at 20 mm depth in a pot (250 mm diameter) containing either autoclaved or nonautoclaved soil. The pots were exposed for seven days at 5°C followed by temperature of 20°C (experiment 1) or at constant temperature of 20°C (experiment 2) in incubators with an eighteen hours photoperiod. During the experiment, enough water (with the same temperature as incubator) was added to the pots to keep them continuously damp. Percentage emergence was calculated in each pot based upon the number of seedlings, when the seedlings were approximately 50 - 70 mm of height.

**Data analysis.** The General Linear Model (GLM) of the SAS program was used for analysis of variance of the data. The least significant difference test (LSD) was used to determine the statistically differences between those means with significant *F-value*.

#### **RESULTS AND DISCUSSION**

The results of germination test showed that there was no significant difference for percentage germination of brown and yellow seeds (Table I), indicating that there was no association between seed colour and germination capability in germination test. Therefore, germination capability of yellow seed is not a problem regarding stand establishment of yellow-seeded genotypes of flax. The finding of no association between seed colour and germination in germination test were similar to the results of Comstock *et al.* (1969), Groth *et al.* (1970) and Saeidi and Rowland (1999a); however, Culbertson *et al.* (1960) and Culbertson and Kommedahl (1956) found that yellow flaxseed had lower germination than brown seeds. The discrepancies among these studies may be explained by the germination differences among different loci conditioning seed colour, the genetic background of the genotypes tested or different conditions of germination tests.

While seed colour had no significant effect on percentage germination in germination test, the effect of seed colour on seed vigour was significant and yellow seeds had significantly lower percentage germination than brown seeds in the vigour test (Table I). Reduction of seed vigour in yellow seeds in this study was in agreement with the results previously obtained by Saeidi and Rowland (1999a). This association between seed colour and seed vigour indicated that microorganisms or other soil constituents might have negative effect on germination of yellow seeds in vigour test (Saeidi & Rowland, 2000).

In the vigour test, the effect of soil type on germination was significant and when seeds were lightly sprinkled with soil from the field, germination was significantly and considerably lower than when no soil was used (Table II). Also, in vigour test, soil autoclaving significantly and considerably improved germination of non-treated seeds with fungicide; however, it had no significant effect on germination of treated seeds (Table III). Also, soil autoclaving significantly improved emergence at constant temperature of 20°C (experiment1) (Table II) and significant interaction between seed colour and type of soil showed that this improvement happened only for yellow seeds (Table V). Since autoclaving is employed as a method for sterilizing growing media, the most likely explanation of increasing emergence of yellow seeds in autoclaved soil is that soil microorganisms were killed in autoclaved soil; however, in non-autoclaved soil, microorganisms would have been present and could have negative effect on germinating seed, killing the developing seedling (Reitz et al., 1947; Kommedahl et al., 1955; Groth et al., 1970).

Significant interaction between seed colour and the type of soil in the vigour test showed that yellow seeds had significantly lower percentage germination than brown seeds in all type of soils, but difference of percentage germination among the two seed types was 12.7%, 21.8% and 59% in conditions of without soil, autoclaved soil and non-autoclaved soil, respectively (Table III). These results showed that there was so much difference between germination capability of brown and yellow seeds when non-autoclaved soil was sprinkled on seeds in vigour test. In comparison to non-autoclaved soil, autoclaved soil significantly and considerably improved germination of yellow seeds in vigour test, with no significant effect on percentage germination of brown seeds (Table III). Also, the

 Table I. Percent germination in germination and vigour test for brown and yellow seeds

| Seed colour | Germination test | Vigour test |  |
|-------------|------------------|-------------|--|
| Brown       | 93.0             | 73.5        |  |
| Yellow      | 91.0             | 42.3        |  |
| LSD(0.05)   | 2.8              | 7.0         |  |

Table II. Percent germination and emergence in soil at constant temperature of 20°C (experiment 1) and at 5°C for 7 days followed by temperature of 20°C (experiment 2) for different type of soil

| Soil type           | Germination in vigour test (%) |      | Emergence (%)<br>in Experiment 2 |
|---------------------|--------------------------------|------|----------------------------------|
| Autoclaved soil     | 46.9                           | 72.0 | 48.7                             |
| Non-autoclaved soil | 41.5                           | 65.8 | 49.4                             |
| Without soil        | 82.3                           |      |                                  |
| LSD(0.05)           | 8.6                            | 5.2  | 5.8                              |

 Table III. Percent germination of brown and yellow

 seeds treated with different fungicide in different soils

| Factor                      | Soil               |                   |                   |                   |  |
|-----------------------------|--------------------|-------------------|-------------------|-------------------|--|
|                             | Non-<br>Autoclaved | Autoclaved        | Without<br>soil   | Mean              |  |
| Seed colour                 |                    |                   |                   |                   |  |
| Brown                       | 71.0               | 60.8              | 88.7              | 73.5ª             |  |
| Yellow                      | 12.0               | 39.0              | 76.0              | 42.3 <sup>b</sup> |  |
| Fungicide                   |                    |                   |                   |                   |  |
| Captan                      | 52.0               | 47.0              | 82.8              | 60.6 <sup>a</sup> |  |
| Carbendazime                | 43.0               | 40.0              | 90.0              | 57.7 <sup>a</sup> |  |
| Control (no fungicide used) | 29.0               | 63.0              | 74.3              | 55.4 <sup>a</sup> |  |
| Mean                        | 41.5 <sup>b</sup>  | 49.9 <sup>b</sup> | 82.3 <sup>a</sup> |                   |  |

For each factor in column and for row, means followed by the same letter are not significantly different at the 0.05 level of probability using the LSD test.

LSD value at 5% level of probability for comparing interaction means between soil and seed colour, and soil and fungicide was 12.2 and 14.0, respectively.

effect of seed colour on emergence was significant and yellow seeds had significantly lower percentage emergence than brown seeds in both experiments of 1 and 2 (Table IV): however, significant interaction between seed colour and type of soil for emergence in experiment1 showed that there was no significant difference between percentage emergence of brown and yellow seeds in autoclaved soil, but in non-autoclaved soil their difference was significant and considerably high (Table V). The same significant interaction has been previously observed in another experiment (Saeidi & Rowland, 2000) in which difference of emergence between yellow and brown seeds in nonautoclaved soil, was much higher than in autoclaved soil. The great effect of seed colour on emergence is consistent with previous experiments in which yellow seeds had lower percentage emergence than brown seeds in both field and growth chamber experiments (Saeidi & Rowland, 1999b & 2000; Culbertson & Kommedahl, 1956). Autoclaving the soil resulted in a significant improvement of emergence only in yellow seeds (Table V). Therefore, it is not seed colour per se that is important, but the interaction between

seed colour and environmental factors such as soil and/or temperature leads to the reduction of emergence in yellowseeded flax, most likely due to the activity of microorganisms. More resistance of brown seeds to soilborn microorganisms might be associated with the presence of phenolic compounds (tannins), which are known to have anti-microbial properties (Scalbert, 1991) and are found as pigments in the seed coat of brown seeds of flax, but are nearly absent in yellow seeds (Freeman, 1995; Oomah *et al.*, 1995). The antimicrobial properties of tannins (phenolic compounds) are therefore the likely reason of seed vigour improvement in brown seeds.

There were no significant differences for percentage germination of the seeds treated with different fungicides in germination test (Table VI); however, the significant interaction between fungicide treatment and type of soil showed that seed treatment significantly improved seed germination of flaxseed, when non-autoclaved soil was used in vigour test and Captan was more effective than Carbendazime (Table III). Captan is a protective, non-systemic fungicide that provides protective control of a wide range of fungal pathogens on the external surface of seeds and is useful in control of damping-off of seedlings (Smith *et al.*, 1999; Hewitt, 1999) and Carbendazime is a systemic

Table IV. Percent germination and emergence in soil at constant temperature of 20°C (Experiment # 1) and at 5°C for 7 days followed by temperature of 20°C (Exp. 2) for brown and yellow seeds

| Seed      | Germination (%) Germination |             | Emergence Emergen |        |             |             |
|-----------|-----------------------------|-------------|-------------------|--------|-------------|-------------|
| colour    | in                          | Germination | (%) in            | Vigour | (%) in Exp. | (%) in Exp. |
|           | test                        |             | test              |        | 1           | 2           |
| Brown     | 92.8                        |             | 73.6              |        | 74.4        | 55.1        |
| Yellow    | 90.6                        |             | 42.4              |        | 63.6        | 43.25       |
| LSD(0.05) | 2.8                         |             | 7.0               |        | 5.2         | 5.8         |

Table V. Percent emergence for brown and yellow seeds in autoclaved and non-autoclaved soil at constant temperature of 20°C (experiment 1)

| Seed colour    | Autoclaved | Non- autoclaved |
|----------------|------------|-----------------|
| Brown          | 74.8       | 73.9            |
| Yellow         | 69.2       | 57.7            |
| LSD (0.05)=7.3 |            |                 |

Table VI. Percent germination and emergence in soil at constant temperature of  $20^{\circ}$ C (experiment1) and at 5°C for 7 days followed by temperature of  $20^{\circ}$ C (experiment 2) for seeds treated with different fungicides

| Fungicide    | Geri | nination (%) | Germination | Emergence | Emergence   |
|--------------|------|--------------|-------------|-----------|-------------|
|              | in   | Germination  | (%) in      | (%) in    | (%) in Exp. |
|              | test |              | Vigour test | Exp. 1    | 2           |
| Captan       | 91.8 |              | 60.6        | 70.8      | 54.2        |
| Carbendazime | 91.5 |              | 57.8        | 69.9      | 47.9        |
| Control      | 91.5 |              | 55.3        | 66.0      | 45.1        |
| LSD(0.05)    | 3.5  |              | 8.6         | 6.3       | 7.1         |
| Mean         | 91.6 |              | 57.9        | 68.9      | 49.6        |

fungicide (Hewitt, 1999).

Seed treatment with fungicide had no significant effect on emergence of flaxseed at constant temperature of 20°C (experiment 1); however, at temperature condition similar to vigour test (experiment 2), seed treatment with Captan significantly increased percentage emergence (Table VI). These results are in agreement with the knowledge that soil microorganisms could cause reduction in emergence of flax and seed treatment with fungicide can increase the emergence, depending on locality, temperature, moisture conditions of the soil and percentage of cracked seeds (Schuster et al., 1943; Kommedahl et al., 1955). Fungicidal seed treatment is an inexpensive method for disease control that can protect the seedlings against a variety of fungal pathogens and improve emergence (Agrios, 1997). An effective seed treatment must eliminate pathogens, but being non-toxic to seeds (Neergaard, 1977). The results of germination test showed that the fungicides used in this study had no phytotoxicity to seeds and were effective to improve seed vigour and emergence (Table VI). This means that with seed treatment, the rate of seeding can be reduced; a factor of considerable economic important when high priced seed is sown.

These experiments confirmed the role of yellow seeds in reducing the seed vigour and emergence of flax, most likely due to the role of microorganisms. Therefore, it is necessary to screen for seed vigour and emergence of yellow-seeded genotypes in breeding programs of flax and/or to use fungicidal seed treatment to protect the seedlings from fungal pathogens and improve seed vigour and emergence.

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