Original Research Article

Effect of Seed Treatment and Seed Borne Mycoflora on Vigour of Mungbean [Vigna radiata (L.) Wilczek] Grown in Agro – Climatic Zones of Chhattisgarh, India

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A B S T R A C T

Mungbean is grown principally for its protein content. Seed borne mycoflora affect the germination and vigour of seeds. Thus, due to seed borne diseases, there is a reduction in the production, resulting in failure of fulfilling the demand of mungbean seeds. The associated seed borne mycoflora were found to reduce the germination and thereby seedling vigour index. Fungicides and Trichoderma were found to increase the vigour index by keeping mycoflora under check and plant growth promoting activities of Trichoderma. Seedling vigour was markedly reduced by some of the seed borne mycoflora when evaluated by seed inoculation techniques. Rhizopus sp. (68.14%) shows overall impact irrespective of seed lots followed by Fusarium sp. (64.05%). Seedling showed initial wilting type symptoms yielded Fusarium sp. and root rot like symptoms yielded Rhizopus sp. upon isolation which was identical to both the fungi detected from mungbean seeds. Hence, Rhizopus sp. and Fusarium sp. were found to be pathogenic to mungbean seeds and seed transmissible in nature. It was also found that Macrophomina sp. reduces the vigour index maximum irrespective of seed lots (56.63%) followed by Penicillium sp. (39.25%) and Fusarium sp. (38.85%) in comparison to control when soil inoculation technique was used.

K e y w o r d s
Mungbean, Mycoflora, Seed treatment, Vigour index

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Introduction

Among the pulses, mungbean is popularly known as green gram or golden gram is one of the most important short duration pulse grown in India. The seeds are highly nutritious as it contains about 23.86% protein, 62.6% carbohydrates, 1.15% fat, 5.27% crude fibre, 3.32% ash besides rich in lysine (436 mg/g). It is also rich in Ca, Fe, and K is a good source of vitamins such as thiamine, niacin and vitamin A. The total area covered under mungbean in India was 30.41 lakh hectares with a total production of 14.24 lakh tonnes. The national yield average was 468 kg/ha. The lowest yield observed in the state of Karnataka (247 kg/ha) followed by Chhattisgarh (269 kg/ha) and Odisha (337 kg/ha) (Anon., 2016).

Contaminated seeds can often result in poor germination and poor seedling vigour, resulting in an un-healthy crop. Field fungus associated with seeds causes deterioration of seed quality, affect viability and reduces germination (Shrivastava and Gupta, 1981). The infected seeds fail to germinate or seedlings and plants developed in the field
from infected seeds may escape early infection but may often be infected at the later stage of growth. Besides, pathogens can spread over a long distance and uninfected field may be infected by the seeds in which different pathogens are present (Fakir et al., 2001).

The seed borne pathogen associated with mungbean seeds externally or internally may caused seed rot, seedling blight and resulting into low germination. Some fungi are associated with testa and cotyledonal of seeds infected in form of mycelium and conidia (spores), after germination the infection transmitted to hypocotyls and stem bases as well as dicotyledonary leaves of seedling.

Some seed borne pathogens having ability to kill the seedling or plants and substantially reduce the productive capacity (Rahman et al., 1999). Seed mycoflora play an important role in determining the quality and longevity of seed.

Mungbean is subjected to several mycoflora which are seed borne, soil borne and air borne. Seed borne mycoflora associated with Mungbean reported include Macrophomina phaseolina, Aspergillus flavus, Colletotrichum sp., Drechslera sp., and Myrothecium sp. These fungi were negative effect on germination and vigour of seeds (Sarita et al., 2014).

Till date, seed health evaluation aspects like mycoflora associated their effect on seedling vigour index, transmission and management not studied well and documented of mungbean grown in all agro-climatic zones of Chhattisgarh. Therefore, an attempt was taken to carry out the present investigation to find out the effect of seed treatment and seed borne mycoflora on vigour of mungbean seeds.

Materials and Methods

Effect of fungicidal seed treatment on seedling vigour of mungbean

To find out the efficacy of seed dressing fungicides on seedling vigour, seed dressing fungicide viz. Bavistin (Carbendazim), Devithiram (Thiram 75% WP), Safal (Carbendazim 12% + Mencozeb 63% WP), Mencozeb (Dithane M-45) and C.G. Tricocap (Trichoderma) were taken at their recommended dose along with a control (without treated). Treated and untreated seeds were grown in pots filled with sterilized soil. The seedling growth was assessed in terms of seedling vigour index 21 days after sowing. The germination percentage, root length and shoot length were recorded to calculate seedling vigour index of each treatment and seed samples. The shoot length measured from the base of the shoot to upper most leaf tip. For measuring the root length, plant was carefully uprooted first, gently washed and carefully placed on clean transparent glass piece. The length of root system was measured from collar region to the end of longest tip. The seedling vigour index was calculated by using the following formula given by Abdul- Baki and Anderson (1973)

\[
\text{Seedling vigour index} = (\text{Mean shoot length} + \text{Mean root length}) \times \text{Germination percentage}
\]

Effect of seed borne mycoflora on seedling vigour by using seed inoculation technique

Apparently healthy surface sterilized (1.0 % NaOCl) mungbean seeds were taken for this study. The seeds were rolled on 7-10 days old sporulating culture of individual mycoflora thriving on PDA in Petri plate. The rolled seeds were grown in pots filled with sterilized soil. The seedling growth was assessed in term of seedling vigour index 21 days after sowing as described earlier.
Effect of seed borne mycoflora on seedling vigour by using soil inoculation technique

All seed borne fungi detected in various methods used were grown separately and multiplied in sterilized wheat grain substrate. Substrate was inoculated with seven days old culture of individual fungus separately. The inoculated substrate was incubated at 25 ± 2 °C for ten days. Substrate was shaken every day to avoid clumping. The pots were filled with sterilized soil and infected by each mycoflora inoculum. For soil inoculation, upper four cm layer of the soil was thoroughly mixed with culture grown in wheat medium @ 10g / pot and watered to just wet the inoculated soil. Pots were kept in glass house for 72 hours for proper soil infection and establishment of mycoflora before sowing of mungbean seeds. Seeds were surface sterilized (1.0 % NaOCl) before sowing followed by three washing with sterile distilled water and were sown in inoculated soil @ 20 seeds per pot. A set of control was also kept with surface sterilized seeds sown in sterilized un-inoculated soil. Pots were watered at regular intervals. The seedling growth was assessed in term of seedling vigour index 21 days after sowing as described earlier.

Results and Discussion

Effect of seed treatment on seedling vigour of mungbean seeds

In glass house condition pot experiment was conducted to know the effect of different fungicides and bio control agent (Trichoderma) on the seedling vigour of mungbean seeds collected from five districts. Fungicide and Trichoderma treated and untreated seeds were sown in pre sterilized soil and observations were recorded for vigour index 21 days DAS. Data presented in table 1(a) shows that all the fungicides and Trichoderma treated seeds had higher vigour index as compare to that of control.

Maximum seedling vigour index was recorded Raigarh district seed lot in C.G. Tricocap (2901.06%) treatment which was 67.26 per cent more than that of control in of Raigarh district seed lot followed by Jagdalpur district in Devithiram (2716.66%) which was 56.44 per cent more than that of control in Jagdalpur district. Least vigour index was recorded in Kawardha district (898.32%) in control (untreated). This might due to presence of seed borne mycoflora in highest frequency which reduces the germination percentage and thereby seedling index. The above result clearly showed that fungicides and bio control agent Trichoderma taken in the study could be able to reduce the mycoflora associated with seeds and thereby increase the vigour index and in untreated seeds vigour index was less than the treated seeds.

Analysis of data presented in table 1(b) reveal that among fungicides, maximum mean increase in vigour index over control (56.61%) was recorded in Mancozeb across the five seed lots treated. Interestingly, Trichoderma treated seeds of five seed lots of mungbean recorded second least mean increase in vigour index over control (50.19%). This might be attributed that Trichoderma not only reduces seed borne mycoflora but also exhibit the plant growth promoting activity and thereby higher vigour index was recorded over control across the five seed lots tested.

It is clear from the above finding that seed borne mycoflora reduces the seedling vigour index whereas fungidal and biocontrol agent Trichoderma increases the seedling vigour index by keeping seed borne mycoflora under check. Similar results were also reported by various researchers while working with seed.
health evaluation agreeing the finding of present study (Teama et al., 2000), Rahman et al., (2002), Singh et al., (2002), Sethuraman et al., (2003), Prajapati et al., (2003), Javaid and Anjum (2006), Rajeshwari and Kumari (2009), Dabbas et al., (2009), Mandhare et al., (2010), Pan et al., (2010), Geetanjali and Giri (2014), Suramwar et al., (2014), Ashwini and Giri (2014 a & b), Kandhare (2014), Singh et al., (2014), Tak et al., (2015), Gawade et al., (2016), Shekhar (2016) and Fatma et al., (2017).

Effect of seed borne mycoflora on seedling vigour by using seed inoculation technique

It is depicted from data presented in table 2(a) and 2(b) that seedling vigour was markedly reduced by some of the seed borne mycoflora when evaluated by seed inoculation technique. Overall impact in reducing seedling vigour index was shown by Rhizopus sp. (68.14%) followed by Fusarium sp. (64.05%) across all five seed lots evaluated as compared to that of control. Maximum reduction in seed lot of Ambikapur district was caused by Rhizopus sp. (92.24%) followed by Fusarium sp. (66.91%) and Penicillium sp. (64.90%). In seed lot of Balod district, reduction was maximum by Rhizopus sp. (44.13%) followed by A. flavus (42.13%) and Fusarium sp. (39.09%). The reduction in seedling vigour index of seed lot of Jagdalpur was maximum by Rhizopus sp. (90.80%) followed by Macrophomina sp. (79.90%) and Fusarium sp. (63.23%). Fusarium sp. caused maximum reduction in seedling vigour index of seed lot of Kawardha and Raigarh districts. (89% and 62.03%, respectively) followed by Rhizopus sp. (70.24% and 43.31%, respectively). Reduction in seedling vigour may be attributed due to inhibition of germination of seed by inoculated mycoflora some of the weak seedling with light green colour leaves and reduced plumule and radical length or wilt like symptoms and rotting of roots of seedling were also noticed.

The underdeveloped symptoms shown by some of the seedling were subjected to isolation of mycoflora from infected plant / root tissues. Isolation from wilted type plant tissue yielded the fungus identical to with the Fusarium sp. which was isolated from seed sample. Similarly, isolation from infected root tissues yielded the fungus identical with the Rhizopus sp. which isolated from seed sample.

| S. N. | Treatment               | Ambikapur | Balod   | Jagdalpur | Kawardha | Raigarh |
|------|-------------------------|------------|---------|-----------|----------|---------|
| 1    | Carbendazim (Bavistin)  | 1922.76    | 2210.08 | 2106.8    | 1086.48  | 1769.88 |
| 2    | Thiram (Devithiram)     | 2351.72    | 2026.24 | 2716.66   | 1099.4   | 2403.28 |
| 3    | Mancozeb+ Thiram (Safal)| 2206.26    | 2434.32 | 2369.84   | 2171.32  | 2374.4  |
| 4    | Mancozeb (Dithane M-45) | 2087.2     | 2346.08 | 2191.56   | 1297.72  | 1774.52 |
| 5    | Trichoderma (C.G.Tricocap) | 2075.7  | 2549.38 | 2519.4    | 1538.22  | 2901.06 |
| 6    | Control                 | 1764.18    | 1704.76 | 1734.3    | 898.32   | 1734.4  |

Table.1a Effect of seed treatment on seedling vigour index of mungbean seeds
### Table 1b Effect of seed treatment on per cent increase over control of mungbean seeds

| S. N. | Treatment                          | Ambikapur | Balod  | Jagdalpur | Kawardha | Raigarh | Mean increase over control |
|------|------------------------------------|-----------|--------|-----------|----------|---------|---------------------------|
| 1    | Carbendazim (Bavistin)             | 8.98      | 29.64  | 21.47     | 20.94    | 2.04    | 16.61                     |
| 2    | Thiram (Devithiram)                | 33.30     | 18.86  | 56.64     | 22.38    | 38.56   | 33.91                     |
| 3    | Mancozeb+ Thiram (Safal)           | 25.05     | 42.79  | 36.64     | 141.70   | 36.90   | 56.61                     |
| 4    | Mancozeb (Dithane M-45)            | 18.30     | 37.61  | 26.36     | 44.46    | 2.31    | 25.80                     |
| 5    | *Trichoderma* (C.G.Tricocap)       | 17.65     | 49.54  | 45.26     | 71.24    | 67.26   | 50.19                     |
| 6    | Control                            | -         | -      | -         | -        | -       | -                         |

### Table 2a Effect of seed borne mycoflora on seedling vigour index by seed inoculation technique

| S.N. | Mycoflora   | Ambikapur | Balod  | Jagdalpur | Kawardha | Raigarh |
|------|-------------|-----------|--------|-----------|----------|---------|
| 1    | *A. flavus* | 1848.6    | 1044.5 | 2148.5    | 2076.1   | 1902.6  |
| 2    | *A. niger*  | 2038.4    | 1608.75| 1864.2    | 1485.0   | 2352.0  |
| 3    | *A. fumigatus* | 1137.15 | 1410.6 | 1394.0    | 994.4    | 1685.2  |
| 4    | *Rhizopus* sp. | 199.0    | 1008.4 | 201.0     | 646.5    | 1447.5  |
| 5    | *Macrophomina* sp. | 1398.0 | 1528.8 | 439.4     | 762.85   | 2157.0  |
| 6    | *Penicillium* sp. | 900.3    | 1448.5 | 2124.2    | 1745.7   | 1637.3  |
| 7    | *Fusarium* sp. | 848.8    | 1099.35| 803.95    | 238.95   | 969.5   |
| 8    | Control     | 2565.5    | 1805.05| 2186.8    | 2172.8   | 2553.75 |
Table 2b Effect of seed borne mycoflora on per cent decrease over control by seed inoculation technique

| S.N. | Mycoflora       | District (decrease over control (%)) | Mean decrease over control |
|------|-----------------|-------------------------------------|---------------------------|
|      |                 | Ambikapur  | Balod   | Jagdalpur | Kawardha | Raigarh |
| 1    | A. flavus       | 27.94     | 42.13   | 1.75      | 4.45     | 25.49   | 31.75   |
| 2    | A. niger        | 20.54     | 10.87   | 14.75     | 31.65    | 7.90    | 18.3    |
| 3    | A. fumigatus    | 55.67     | 21.85   | 36.25     | 54.23    | 34.01   | 35.27   |
| 4    | Rhizopus sp.    | 92.24     | 44.13   | 90.80     | 70.24    | 43.31   | 30.64   |
| 5    | Macrophomina sp.| 45.50     | 15.30   | 79.90     | 64.89    | 15.53   | 56.63   |
| 6    | Penicillium sp. | 64.90     | 19.75   | 2.86      | 19.65    | 35.88   | 30.26   |
| 7    | Fusarium sp.    | 66.91     | 39.09   | 63.23     | 89.00    | 62.03   | 38.85   |
| 8    | Control         | -         | -       | -         | -        | -       | -       |

Table 3a Effect of seed borne mycoflora on seedling vigour index by soil inoculation technique

| S.N. | Mycoflora       | District (seedling vigour index) |
|------|-----------------|----------------------------------|
|      |                 | Ambikapur | Balod | Jagdalpur | Kawardha | Raigarh |
| 1    | A. flavus       | 1643.4    | 1327.7 | 1659.6   | 985.05   | 2110.55 |
| 2    | A. niger        | 2161.6    | 1803.6 | 2094.4   | 1103.4   | 1993.8  |
| 3    | A. fumigatus    | 1480.05   | 1459.15| 1494.0   | 1764.1   | 910.8   |
| 4    | Rhizopus sp.    | 1554.0    | 1506.7 | 1757.7   | 1512.6   | 1344.6  |
| 5    | Macrophomina sp.| 1525.5    | 1342.0 | 413.7    | 374.1    | 1197.0  |
| 6    | Penicillium sp. | 1273.05   | 1794.0 | 1319.85  | 657.45   | 1630.2  |
| 7    | Fusarium sp.    | 2140.8    | 1684.2 | 980.65   | 778.95   | 1230.6  |
| 8    | Control         | 2565.5    | 1805.05| 2186.8   | 2172.8   | 2553.75 |
Table 3b: Effect of seed borne mycoflora on per cent decrease over control by soil inoculation technique

| S.N. | Mycoflora       | District (increase over control) | Mean decrease over control |
|------|----------------|---------------------------------|----------------------------|
|      |                | Ambikapur | Balod | Jagdalpur | Kawardha | Raigarh |            |
| 1    | *A. flavus*    | 35.94     | 26.44 | 24.10     | 54.95    | 17.35   | 31.75       |
| 2    | *A. niger*     | 15.74     | 0.08  | 4.22      | 49.54    | 21.92   | 18.3        |
| 3    | *A. fumigatus* | 42.30     | 19.16 | 31.24     | 19.32    | 64.33   | 35.27       |
| 4    | *Rhizopus* sp. | 39.42     | 16.52 | 19.10     | 30.83    | 47.34   | 68.14       |
| 5    | *Macrophomina* sp. | 40.53 | 25.65 | 80.96     | 82.89    | 53.12   | 56.63       |
| 6    | *Penicillium* sp. | 50.37 | 0.61  | 39.25     | 69.93    | 36.16   | 39.26       |
| 7    | *Fusarium* sp. | 16.55     | 6.69  | 54.86     | 64.37    | 51.81   | 64.05       |
| 8    | Control        | -         | -     | -         | -        | -       | -           |

Hence, it was proved that the detected seed borne mycoflora namely *Fusarium* sp. and *Rhizopus* sp. were pathogenic to mungbean seed and observed seed transmissible in the present study. Brayford (1996) also observed that *Fusarium solani* transmitted via seed infected planting material or movement of soil. Rawal and Singh (2015) reported that, out of twelve seed borne mycoflora of opium poppy, three fungi *A. alternata*, *R. solani* and *Fusarium solani* were found pathogenic and seed to plant transmissible in nature.

Effect of seed borne mycoflora on seedling vigour by using soil inoculation technique

Soil inoculation technique was used to know the effect of seed borne mycoflora on seedling vigour index and data presented in table 3(a) and 3(b). It was clear from the table that *Macrophomina* sp. reduces the vigour index maximum irrespective of seed lots (56.63%) followed by *Penicillium* sp. (39.25%) and *Fusarium* sp. (38.85%) in comparison to control. In Ambikapur seed lot, maximum reduction in seed lot was recorded by *Penicillium* sp. (50.37%) followed by *Macrophomina* sp. (40.53%). *Aspergillus flavus* reduces maximum vigour index (26.44%) followed by *Macrophomina* sp. (25.65%) in Balod district seed lot. In Jagdalpur district seed lot, maximum reduction in seedling vigour index was recorded in *Macrophomina* sp. (80.96%) followed by *Fusarium* sp. (54.86%). *Macrophomina* sp. reduces maximum vigour index (82.89%) followed by *Penicillium* sp. (63.93%) in Kawardha district seed lot. In Raigarh district seed lot, reduction in seedling vigour index was maximum in *A. fumigatus* (64.33%) followed by *Macrophomina* sp. (53.12%).

These methods being quick and simple can be used in routine pathogenicity tests of fungal pathogens, both the methods are of equal importance.

It was observed that these fungi reduced the per cent seed germination and seedling vigour as reported by different workers (Prasad, 1983;
Paul, 1992). Many plant pathogenic fungi are known to produce phytotoxic metabolites (Vidhyasekaran et al., 1970). Importance of the production of such toxic metabolites is more obvious when the pathogen is seed borne because it may either inhibit seed germination or adversely affects the initial growth of seedlings.

It was observed that seed borne mycoflora inhibit the germination of mungbean seeds and retarded the growth of seedling may be due to production of metabolites. Similarly, Gandhi and Raghuchander (2001) also observed the effect of seed borne fungi on seed germination and seedling vigour of coriander. The reduction of seed germination and seedling vigour may be attributed to absorption, translocation and interference of toxic metabolites with routine biochemical activities of the seed and seedling during germination and growth of the seedling as evident by the work of Jain et al., (1996), and Prokinova and Buresova (1996).

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