Minimization of floret infection by fungi causing grain mold in sorghum (*Sorghum bicolor*) through use of fungicides

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ABSTRACT: Field experiments were conducted with four popular grain sorghum (*Sorghum bicolor* L.) cultivars that included two each of varieties and hybrids to evaluate the efficacy of fungicides for minimization of floret infection by grain mold fungi during two successive rainy seasons of 2012 and 2013 at Hyderabad. The spraying of fungicides (Propiconazole or Mancozeb) at 80% anthesis significantly reduced seed-borne infection at milk stage and grain mold severity at maturity. Propiconazole was found to be the most effective fungicide that reduced fungal infection frequency and grain mold severity in sorghum. It reduced 65% of *Fusarium*, 89% of *Curvularia* and 67% of total fungal infection at milk stage compared to the control. The study will provide valuable information for resistance breeding programme against specific grain mold pathogen.

Key words: *Curvularia*, fungicide, *Fusarium*, grain mold, sorghum

The grain mold fungi infect at flowering stage (Bandyopadhayay, 1986; Forbes *et al.*, 1992; Das *et al.*, 2012b). Castor and Frederiksen (1977) reported that principal grain mold fungi were pathogens and its problem was because of pathogens. Little and Magill (2009) reported that grain mold fungi (*Fusarium thapsinum*) reduces formation of caryopsis in sorghum (*Sorghum bicolor* L.). These fungi behave as pathogen or parasite depending on host susceptibility and prevailing weather. On highly susceptible genotype, 296B, they behave as pathogen and extensively invade immature grains, causing premature seed rotting under post-flowering humid conditions (DSR, 2010; Das *et al.*, 2013). However, when post-flowering weather is dry and genotype is tolerant to grain mold, premature seed rot symptom is not evident.

Fungi, in such case, most probably harbor in hilar region of seed much like a parasite without causing damage to the grain. Such grains though look healthy, produce fungal growth on incubation in laboratory (Fig. 1). When environmental conditions are appropriate, mycelial growth pushes through the pericarp, producing a fungal mass which can completely cover the grain causing grain mold (Glueck and Rooney, 1980). Rainy season sorghum in India is regularly affected by grain mold incurring heavy losses to the farming community. Estimated annual loss in India is around US$ 50-80 million depending on incidence and severity while average loss per unit area is around ₹ 2323/ha (Das and Patil, 2013).

Other group of grain mold fungi is related to grain weathering, which occurs in mature grains on exposure to prolong wet weather. Contrary to fungi that infect sorghum floret, fungi causing grain weathering are saprophytes and their occurrence is extremely weather dependant. Fungicides have been used for management of grain weathering but there is no report to know the effect of fungicide application on infection of sorghum floret or tender grain. Previous studies reported that a combination of Captan (0.2%) + Aureofungin (200 ppm), followed by Mancozeb (0.27) + Captan (0.2%), and then Thiram (0.2%) + Carbendazim (0.5%) were useful for reduction of grain weathering (Anahosur, 1992). Fungicidal sprays have been recommended since long but they have not been adopted widely.

Unfavorable benefit: cost ratio because of low cost of sorghum grain was a hindrance (Somani *et al.*, 1995; Bandyopadhayay *et al.*, 2000). However, recent increase in prices of all grains including sorghum might have given a scope for reassessment of previous thinking. In this study, focus was on evaluation of efficacy of fungicides for minimization of floret infection by grain mold fungi. Recent studies have shown that frequency of infection at milk stage grain in popular Indian cultivars was about 25% with natural inoculums and grain infection frequency

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at milk stage played significant role in deciding grain mold severity on post-maturity sorghum (Das et al., 2014).

**MATERIALS AND METHODS**

**Field experiments**

Field experiments were conducted at Directorate of Sorghum Research, Rajendranagar, Hyderabad, during rainy season of 2012 and 2013. Hyderabad is a hot spot of grain mold that occurs in moderate to severe form every year. The trial was laid out in a split plot design using spray treatments as main plot and cultivars as sub-plot with four replications in a grain mold screening block. Four popular rainy season grain sorghum cultivars including two each varieties (CSV17 and CSV20) and hybrids (CSH16 and CSH23) were used in the experiment. They are among popularly grown recent sorghum cultivars in India. All cultivars have semi-compact panicles with bold grain. The CSV17 is a short height (140 cm) early-maturing (97 days) variety, while others are medium height cultivars (180-240 cm) that mature in around 110 days (Table 1).

Sowing and harvesting was done in June and October respectively. Four cultivars each with single row of 4m length, at a 60 cm row spacing and a plant spacing of 10-15 cm within rows were grown in main plot. Standard crop management practices were followed. Eight uniform flowering plants in each row were labelled for spray treatment and for further observation. The labelled panicles in each row were sprayed with definite treatment on the day of 80% anthesis. Two systemic fungicides, Propiconazole (Propiconazole 25% EC, Syngenta India Ltd.) @ 0.1% a.i.; Carbendazim (Carbendazim 50% WP, BASF India Ltd) @ 0.2% a.i; and one contact fungicide Mancozeb (Mancozeb 75% WP, Insecticides India Ltd.) @ 0.2% a.i were used as fungicial treatments.

Spray of sterile water served as the control. Spraying was done using aerosol spray bottles. Proper care was taken for uniform spraying of panicles, avoiding any drifting of fungicial spray to neighboring cultivars. Each panicle was covered fully with moistened paper bag (42 cm × 16 cm × 6 cm) immediately after spray for two days. This helped to keep humid microenvironment surrounding panicle and to promote infection by natural air-borne inoculum that might have settled on panicle. Paper bags were removed from the panicles after two days. During flowering to physiological maturity (i.e. August and September) the experiments received enough rainfall and high relative humidity (>87%) during both years (Fig. 2).

**Assay for fungal infection at milk stage**

Eight to ten days old tender grains (milky stage) from each treatment were used for assessing floret infection. At milk stage (MS) seed is tender, soft, and a white milk-like liquid is obtained when seed is squeezed. Milk stage seed was selected as this was the earliest possible stage when seed could be sampled without much damage. Second sampling of seed from each treatment was done at physiological maturity (PM) (40-45 days after flowering depending on cultivar) which was judged by black layer on seed at the hilar end. Panicle branches containing seed were randomly sampled from all parts of panicle (base, middle and top). Seeds were carefully separated from panicle branches without causing damage. Seed samples from eight labelled panicles in a cultivar were pooled to form one replicate. Samples were used for isolation of fungi in a laboratory.

Assay for seed-borne fungal infection was done as described by Das et al. (2012a). One hundred seeds from each replication were plated to study seed-borne fungi. Seeds were washed twice with sterile distilled water, surface-sterilized using 4% solution of sodium hypochlorite (NaOCl) for five minute, and then rinsed thrice with sterile distilled water. The seeds were then air-dried in a laminar flow hood. Twenty-five surface sterilized seeds were placed on sterile, wet filter paper in a Petri-dish humidity chamber. Petri-dish humidity chambers were prepared by lining the lower lid with a thin layer of absorbent cotton followed by two layers of blotter paper. The cotton-blotting papers were wetted with...
10 ml of distilled water. Seeds were incubated at 28 ± 1°C in a BOD incubator with 12 h alternate cycles of light and dark for 5-8 days. Sterile water was added in Petri-dishes when required to keep the paper moisten for promoting fungal growth. Incubation period for MS and PM seeds were 8 and 5 days respectively to promote growth of seed-borne fungi.

Tender seeds contain more antifungal phenolic compounds and therefore might need more time for fungi to grow (Menkir et al., 1996). After incubation, samples were observed for number of seeds infected with fungi using a magnifying glass. Grains showing even slight growth of fungus were counted as positive for fungal growth. Infection percentage was calculated on 100 grains from 4 Petri-dishes. Identification of seed-borne fungi was done by microscopic observations following procedures described in fungus identification manuals (Ahmed and Ravindra Reddy, 1993; Leslie and Summerell, 2006).

Grain mold severity
Grain mold severity was scored on mature grains two times following 1-9 visual scale, where 1= all grains are free from mold infection and 9= more than 75% grains are mold infected (Audilakshmi et al., 2011). First score was recorded on individual panicle in the field at post-physiological maturity (post-PM seed). It was termed panicle grain mold score or PGS. Crop was harvested immediately after taking PGS. Each labelled panicle was threshed separately and scored for threshed grain mold score or TGS. Mean score of 5-8 panicles (a few labelled panicles were damaged and were not considered) formed one replicate.

Data analysis
Data were analyzed using statistical software (Statistix, version 8.1). Variance for individual year was analyzed separately to test the significance of differences among different parameters. The error variances in the field experiment conducted in two years were heterogeneous, as revealed by Bartlett’s test (Bartlett, 1937). Therefore, two years data were not combined for analysis. ANOVA for the per cent seed infection by different fungi over different seed development stages, PGS and TGS was performed year wise separately. Arc sine transformations of original data for percentage of seed infection by Fusarium, Curvularia, and total infection were carried out. All pair-wise comparisons of means were done by using Tukey HSD method.

RESULTS AND DISCUSSION
Analysis of variance revealed that fungicidal treatment had a large effect on all the parameters (Table 2). Cultivar had a large effect on all the parameters (except Fusarium infection at MS during 2012, and Curvularia infection at PM during 2013). Significant interactions between treatments and cultivars for fungal infection were noted at MS and PM. Significant mean squares for treatment and cultivar suggested that treatment and cultivar largely varied for most of the characters. Significant interaction effect between treatment and cultivar indicated that treatment might have differential effects on cultivar. Variable weather during different days of application of treatments and genotypic difference for plant height and flowering time might have contributed in the treatment x cultivar interaction.

In this study, fungicides were sprayed at 80% anthesis (flowering) of a cultivar. There was difference of around 15 days between flowering time of the earliest (CSV17) and the latest (CSV20) cultivar (data not shown) during both seasons. On a few occasions rain came the same day after spray that might have caused variation in microenvironment inside the bag and effect of treatments on fungal infection. Influence of weather, such
as relative humidity and temperature on infection by fungi and grain mold development has been well documented (Indira and Muthusubramanian, 2004; Navi et al., 2005).

Fungicidal spray at 80% anthesis showed notable effects on seed-borne infection of sorghum grains at milk and physiological maturity. Results showed similar trends during rainy seasons of 2012 (Table 3) and 2013 (Table 4). All fungicidal treatments significantly reduced seed-borne infection of Fusarium, Curvularia and total fungi at milk stage compared to the control during both seasons. Apart from Fusarium and Curvularia, other fungi that were isolated at milk stage included Alternaria and Bipolaris (data not shown) in very low frequency (<1.0% of total infection). Propiconazole spray caused maximum reduction of all types of seed-borne infection at MS and PM. Reduction of infection at MS was 72.7% and 57.0% of Fusarium, 90.2% and 87.9% of Curvularia and 74.8% and 59.3% of total infection compared to the control during 2012 and 2013. It was 20.5% and 30.1% of Fusarium, 19.9% and 67.3% of Curvularia and 22.6% and 19.7% of total infection at PM compared to the control during 2012 and 2013. Spray of carbendazim significantly reduced all types of seed-borne infections at milk stage. Reductions were 33.0% and 15.2% of Fusarium, 56.0% and 74.7% of Curvularia and 38.4% and 37.6% of total infection compared to the control during 2012 and 2013 respectively. Its effect of reducing infection at PM was not consistent over seasons. Spray of Mancozeb significantly reduced all types of seed-borne infections at MS during both seasons. Reductions were 65.7% and 36.1% of Fusarium, 69.7% and 73.7% of Curvularia and 64.0% and 38.1% of total infection compared to the control during 2012 and 2013. Its effect of reducing infection at PM was not consistent over seasons. Spray of Mancozeb significantly reduced all types of seed-borne infections at MS during both seasons. Reductions were 65.7% and 36.1% of Fusarium, 69.7% and 73.7% of Curvularia and 64.0% and 38.1% of total infection compared to the control during 2012 and 2013. However, its effect of reducing infection at PM was not consistent over seasons especially for Curvularia and total infection. It significantly reduced Fusarium infection at PM during both seasons.

Enormous literature is available on chemical control of grain mold. Few studies reported about the efficacy of fungicidal spray on grain mold severity (Bandyopadhyay et al., 2000). Many studies dealt with the efficacy of seed dressings for improving seedling emergence and vigor (Munghate and Raut, 1982; Gopinath and Shetty, 1992). So far there was no such report that specified role of fungicidal spray on fungal infection frequency at tender seed (milk stage seed). This study first time reports that spray of Propiconazole or Carbendazim or mancozeb at the time of flowering significantly reduce seed-borne fungal infection at milk stage. Earlier histopathological studies showed that initial infection by grain mold fungi occurs on the apical ends on the spikelet tissues followed by infection of ovary wall (Forbes et al., 1992). Application of fungicide might have affected fungal growth inside the spikelet tissues and thereby reduced infection.

Higher efficiency of propiconazole was due to low LC50 value of propiconazole for fungal spore germination as compared to other two chemicals (personal communication, data not shown). Propiconazole also significantly reduced grain mold score (PGS and TGS) at post-PM during both seasons (Tables 3 and 4). Carbendazim significantly reduced grain mold scores at post-PM during 2013 but its effect was non-significant during 2012. Mancozeb had little effect on post-PM grain mold severity. Reduction of MS infection, eventually contributed to decrease of post-PM grain mold severity because of existence of strong interrelationship (Das et al., 2014).

Frequency of seed infection by Fusarium, Curvularia, and total infection varied among cultivars during both seasons. CSV20 showed significantly less infection of Fusarium (15.0% and 10.9%), Curvularia (2.3% and 3.4%) and total fungi (19.3% and 14.3%) on MS seed than other cultivars (Tables 3 and 4). CSV17 recorded significantly higher infection of all types of fungi at MS during 2013. Cultivars were not different for infection of physiologically mature seed during 2013 but they were during 2012. Total fungal infection at PM was least on

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### Table 3. Efficacy of fungicidal sprays at anthesis on natural infection frequencies of sorghum seed by fungal pathogens at different stages of grain development and post-PM grain mold severity during rainy season, 2012

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fungal infection (%)</th>
<th>Post-PM mold severity (1-9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk stage</td>
<td>Physiological maturity</td>
</tr>
<tr>
<td></td>
<td>Fusarium spp.</td>
<td>Curvularia spp.</td>
</tr>
<tr>
<td>Propiconazole (0.1% a.i.)</td>
<td>16.1(8.2)*c†</td>
<td>4.1(1.3)c</td>
</tr>
<tr>
<td>Carbendazim (0.2% a.i.)</td>
<td>25.6(20.1)b</td>
<td>11.6(5.8)b</td>
</tr>
<tr>
<td>Mancozeb (0.2% a.i.)</td>
<td>17.8(10.3)c</td>
<td>11.7(4.0)b</td>
</tr>
<tr>
<td>Control</td>
<td>32.6(30.0)a</td>
<td>20.4(13.2)a</td>
</tr>
<tr>
<td>Cultivars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSV17</td>
<td>24.5(18.4)a</td>
<td>12.6(5.1)b</td>
</tr>
<tr>
<td>CSV20</td>
<td>20.8(15.0)a</td>
<td>6.4(2.3)c</td>
</tr>
<tr>
<td>CSV16</td>
<td>23.9(17.5)a</td>
<td>16.6(9.8)a</td>
</tr>
<tr>
<td>CSV23</td>
<td>23.1(17.6)a</td>
<td>12.3(7.0)b</td>
</tr>
</tbody>
</table>

*Figures in parentheses are original values from which Arc sine values are derived; †Means with different letters within the same column differ significantly at p< 0.05; Grain mold severity was measured as grain mold score on a scale of 1-9, where 1 = all grains free from mold and 9 = >75% grains are mold infected.*
CSV17 during both seasons. Of the four cultivars CSV20, CSV16 and CSH23 were medium in height and were known for tolerance to grain mold (Tonapi et al., 2009). Relatively better performance of CSV20 and CSV16 for grain mold resistance over other two cultivars was because of their inherent mold resistance. CSV17 was a short heighted early maturing cultivar released for low rainfall areas of North West India. It was not specifically bred for grain mold resistance and therefore its tolerance to grain mold was relatively low. That is why CSV17 recorded significantly higher infection on MS seed than other cultivars.

Short height and earliness also might have contributed to mold susceptibility of CSV17. Despite prevalence of favourable weather for grain mold development mean severity was low (<5.7) as mold tolerant sorghum cultivars were used in present study. It can be noted that total fungal infection at PM was around 80% (80% infections equals to mold score of 9.0) but visual mold score was around 5.7. This was because infection on mature seed was determined by laboratory test on moist Petri plate under which invisible minor infection on seed get expressed as fungal growth, and infection get counted. Grain mold scores significantly varied among cultivars. CSV20 (PGS 5.5 and 3.7; TGS 5.1 and 3.3) and CSV16 (PGS 5.5 and 4.1; TGS 5.0 and 4.5) recorded significantly low grain mold score than CSV17 and CSH23 during both the seasons.

It was summarized that spraying of fungicides at 80% flowering significantly lessened seed-borne fungal infection at milk stage and grain mold severity on mature grain. Propiconazole was found to be one of the most effective fungicide in lessening fungal infection and grain mold severity. Further confirmation of these observations using more number of cultivars at different sorghum regions in India will provide valuable information. Such information might widen knowledge base for resistant breeding program against specific fungal component of grain mold.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fusarium spp.</th>
<th>Curvularia spp.</th>
<th>Total fungi</th>
<th>Fusarium spp.</th>
<th>Curvularia spp.</th>
<th>Total fungi</th>
<th>PGS</th>
<th>TGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSV17</td>
<td>37.4(37.7)a</td>
<td>12.3(5.5)a</td>
<td>42.6(46.3)a</td>
<td>39.9a</td>
<td>24.0a</td>
<td>77.8b</td>
<td>4.6a</td>
<td>4.8a</td>
</tr>
<tr>
<td>CSV20</td>
<td>16.2(10.9)b</td>
<td>9.0(3.4)ab</td>
<td>16.4(14.3)c</td>
<td>41.4a</td>
<td>21.7a</td>
<td>80.6b</td>
<td>3.7c</td>
<td>3.3c</td>
</tr>
<tr>
<td>CSH16</td>
<td>19.9(12.8)b</td>
<td>8.1(4.0)b</td>
<td>23.6(19.7)bc</td>
<td>46.9a</td>
<td>25.7a</td>
<td>85.0b</td>
<td>4.1bc</td>
<td>4.5b</td>
</tr>
<tr>
<td>CSH23</td>
<td>20.5(15.3)b</td>
<td>7.2(2.7)b</td>
<td>29.0(25.3)b</td>
<td>47.3a</td>
<td>25.2a</td>
<td>91.4a</td>
<td>5.0a</td>
<td>5.5a</td>
</tr>
</tbody>
</table>

*Figures in parentheses are original values from which Arc sine values are derived; †Means with different letters within the same column differ significantly at p< 0.05; Grain mold severity was measured as grain mold score on a scale of 1-9, where 1 = all grains free from mold and 9 = >75% grains are mold infected

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