

MAIZE SEED STORAGE MYCOFLORA IN PAKISTAN AND ITS CHEMICAL CONTROL

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Abstract

Eleven seed-borne fungal species were recorded in the 18 maize seed samples collected from 6 maize growing districts of Punjab and Khyber-Pakhtoonkhwa, Pakistan. *Aspergillus flavus*, *A. niger* and *Fusarium moniliforme* were the most prevalent species with the incidence range up to 94, 62 & 43%, respectively. Maximum number of fungal species i.e., 10 was observed from Okara, Sahiwal and Nowshera, 9 from Mansehra, whereas 8 were recorded from Mardan and Pakpattan samples. A comparative study was designed to evaluate eight fungicides to control maize seed mycoflora at the rate of 2.0, 2.5, 3.0 and 3.5 g kg⁻¹. Benlate followed by Thiophanate Methyl and Thiram provided the maximum fungal control at the dose of 3 and 3.5 g kg⁻¹. Maximum germination percentage was attained in case of seed dressing with Benlate followed by Thiram and Thiophanate methyl at the rate of 3g kg⁻¹, which revealed Benlate to be a broad spectrum seed dressing fungicide with no adverse effect on germination.

Introduction

Maize is the third major cereal crop in Pakistan after wheat and rice (Anon., 2011), whereas second in the world (Anon., 2010). Due to its multiple uses, it occupies a prominent position in the agro-based economy of Pakistan. Seed is the vital input for crop production process. Seed health maintenance during different operations, especially, storage is very essential. Fungal microflora is considered major seed deterioration factor in storage. It affects seed viability, germination and also produces mycotoxins which is hazardous for human and animal health (Miller, 1991; Siddiqui & Zaman, 2004). Major maize seed storage mycoflora include *Aspergillus niger*, *Aspergillus flavus*, *Fusarium moniliforme*, *Helminthosporium* sp., *Alternaria* sp., *Rhizopus* sp., and *Penicillium* sp., (Arinze & Sokirko, 1986; Sitara & Akhtar, 2007). Pathogenic fungi including *Cephalosporium acremonium*, *Drechslera* sp., *Macrophomina phaseolina*, *Colletotrichum graminicola*, *Fusarium moniliforme*, *Helminthosporium* sp., *Alternaria* sp., *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* sp., cause different rots, smuts, blights and rusts in maize (Anon., 2004).

Despite decades of research, fungal infection remains a challenging seed problem (Munkvold, 2003). Chemical control of fungal infection is becoming an efficient, economical and reliable method through seed treatment. Siddiqui and Zaman (2004) have reported successful management of seed borne fungi by fungicidal pre-treatments. It is the convenient and inexpensive method to control seed-borne fungi by chemical fungicides. An ideal seed treatment must be highly efficient against the pathogen, safe to the seed, environment and machinery, economical and easy to use (Desai, 2004). Yet it is difficult to find a chemical that fulfils all requirements of an ideal seed treatment. Work done to find out most suitable seed treatment and its dose for different crops, under different conditions and areas is diversified.

Metalaxyl plus mancozeb and Dithane M-45 (Mancozeb) has been reported by Javaid *et al.*, (2006) as the most effective in cereals. Sitara & Akhtar (2007) have also reported Ridomyl Gold (Metalaxyl plus mancozeb)

and Aliette 80 WP (Aluminium fosetyl) as more efficient in contrast to other chemical and organic remedies for maize seed treatment. Other fungicides recommended for broad spectrum seed treatment include Benlate (Bhutta *et al.*, 2004), Carbendazim (Singh *et al.*, 2006), Thiophanate methyl (Bowen *et al.*, 2000) and Thiram (Thomas & Sweetingham, 1999).

Some reports for positive effect of various fungicides on germination include Topsin M (Pathan *et al.*, 2004), Ridomyl Gold (Sitara & Akhtar, 2007) and Fludioxonil (Akgul *et al.*, 2011) whereas some researchers reported for no positive effect of Thiram and Captan on germination (Desai, 2004). The objective of this study was to find out mycoflora associated with maize seed during storage in the major maize growing areas of Pakistan and also to recommend some effective chemical control with no adverse effect on germination.

Materials and Methods

The study comprised two parts viz., maize mycoflora survey in Pakistan and comparison of fungicides to control it.

Maize seed mycoflora in Pakistan: A total of 18 farmers saved seed samples of maize, harvested in same season, of different varieties were collected as per ISTA (Anon., 2004) procedure, from six maize growing districts i.e., Okara, Sahiwal and Pakpattan of Punjab and Mardan, Nowshera and Mansehra of Khyber-Pakhtoonkhwa province. The samples were stored at 5°C before further procedure. All the seed samples were surface sterilized with application of sodium hypochlorite (1%). For detection of seed borne mycoflora, standard blotter method was used (Anon., 2004). In all seed treatments four hundred seeds were placed on three layered water soaked blotter papers in 90 mm Petri plates and the temperature was maintained at 20± 2°C. The seeds were left for incubation with alternating cycles of 12 hours day and night for 7 days under fluorescent light.

Comparison of fungicides: Maize seed infected with *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Fusarium moniliforme*, *Alternaria alternata*, *Drechslera* sp., *Rhizopus arrhizus* and *Penicillium* sp., was treated with eight fungicides viz., Benlate, Carbendazim, Mancozeb, Thiram, Metalaxyl, Thiophanate Methyl, Fosetyl Aluminium and Dimethomorph at the rate of 2.0, 2.5, 3.0 and 3.5 g kg⁻¹. Hundred seeds after weighing were soaked in measured volume of distilled water for 10 minutes and then unit volume of water imbibed by the seeds was calculated. Now solutions for each fungicide with four application rates were prepared for seed treatment.

Fungal identification during survey as well as fungicides comparison after incubation was done based on the characteristics of fungal morphology following authentic taxonomic keys (Von-Arx, 1981; Barnett & Hunter, 1998; Mathur & Kongsdal, 2003). For species identification, monograph of each genus was used. Results were expressed in percentages. Viability of seeds for the samples was evaluated with the AOSA method (Anon., 1990). Counts of germinated seeds were recorded daily, since first day of imbibition up to the achievement of final germination. Data were expressed in terms of germination percentage (GP).

The experiment was laid out in completely randomized design with three replications. The data were subjected to statistical analysis using Costat computer package (CoHort Software, Berkeley, CA, USA). Least significant difference (LSD) test was applied to compare the treatment mean values. Graphical presentation of data was carried out by using the Microsoft Excel program. Comparison of treatment mean values and standard errors also were computed using Microsoft Excel program (Microsoft Corporation, Los Angeles, CA, USA).

Results and Discussion

Maize seed mycoflora in Pakistan: A total of 11 fungal species were detected from 18 stored maize seed samples collected from 6 maize growing districts of Punjab and Khyber-Pakhtoonkhwa, Pakistan. The species isolated were *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Fusarium moniliforme*, *Alternaria alternata*, *Macrophomina phaseolina*, *Drechslera* sp., *Cladosporium* sp., *Rhizopus arrhizus*, *Penicillium oxalicum* and *Penicillium* sp. Maximum number of fungal species i.e., 10 was observed from Okara, Sahiwal and Nowshehra, 9 from Manshehra, whereas 8 were recorded from Mardan and Pakpattan samples. (Fig. 1 A-F). *A. flavus*, *A. niger* and *Fusarium moniliforme* have been the most prevalent fungal species in all the samples as reported in maize seed by Fandohan *et al.*, (2003), Bhutta *et al.*, (2004) and Aksun (2006). The highest incidence record was of *A. flavus* (94%) from Sahiwal followed by *A. niger* (62%) and *Fusarium moniliforme* (43%) from Okara. *Macrophomina phaseolina*, *Drechslera* sp., *Cladosporium* sp. and *Rhizopus arrhizus* exhibited low incidence percentage while, *A. fumigatus*, *Alternaria alternata*, *Penicillium oxalicum* and *Penicillium* sp. revealed intermediate range of occurrence (Fig. 1 A-F). Ghiasian *et al.*, (2004), during a survey of different provinces of Iran, had found

predominance of *Fusarium* species followed by *Aspergillus*, *Rhizopus*, *Penicillium*, and *Mucor* in maize seed. Sitara & Akhtar (2007) have also observed maize seed fungal spectrum partially similar to the present findings including *A. niger*, *A. flavus*, *A. fumigatus*, *Rhizopus* sp., *Drechslera* sp., and *F. moniliforme*. Similarly in lentil seed, *A. niger*, *A. flavus* and *A. fumigatus* have been reported as major and common fungal species (Rahim *et al.*, 2010). These findings reveal vast fungal species spectrum that may be a threat for maize seed during storage as well as in the field.

Comparison of fungicides: Data regarding efficacy of eight seed dressing fungicides expressed varied response for fungal elimination in maize seed lots. Similarly, seed viability was also affected by different fungicides at various doses. All eight fungicides reduced the seed borne fungi with different doses as compared to control. Fungal species observed in control treatment included *A. flavus*, *A. niger*, *A. fumigatus*, *Fusarium moniliforme*, *Alternaria alternata*, *Drechslera* sp., *Rhizopus arrhizus* and *Penicillium* sp. Benlate, Thiophanate Methyl and Thiram provided the maximum fungal control at 3 and 3.5 g kg⁻¹, whereas, Fosetyl aluminium and Metalaxyl were least effective at all the doses as compared to fore-mentioned fungicides. Carbendazim and Mancozeb were found intermediate in effect at 3.5 g kg⁻¹. Dimethomorph failed to eliminate any of the fungal species completely, even at highest dose of 3.5 g kg⁻¹. Among the mycoflora, *Aspergillus niger* exhibited maximum prevalence followed by *A. flavus* and *A. fumigatus*, revealing varied resistance against the tested fungicides. Other fungi including *Alternaria alternata*, *Penicillium* sp., *Rhizopus arrhizus*, *Drechslera* sp., and *Fusarium moniliforme* were found in lower proportions indicating the efficacy of fungicides against these fungi at different application rates. Regarding dosage effect, Benlate expressed maximum efficacy at 3 as well as 3.5 g kg⁻¹ by completely eliminating six fungal species, simultaneously depressing the prevalence of other two species down to 1.3-1.7%. Thiophanate methyl arrested growth of five fungal species at 3.5 g kg⁻¹. Thiram was completely effective against two fungal species at 3 & 3.5 g kg⁻¹. Carbendazim provided maximum control at 3 g kg⁻¹ whereas at higher dose of 3.5 g kg⁻¹ proved to be negative in effect with increased flora. Mancozeb, Fosetyl aluminium and Metalaxyl plus mancozeb suppressed all the fungi at 3.5 g kg⁻¹. Dimethomorph was almost non effective to eliminate the fungi (Fig. 2A-H).

The germination percentage (GP) being the measure of seed viability indicates its ability to emerge and grow. The effect of dosages of various fungicides was pronounced with respect to seed germination (Fig. 3). Fungicides application gradually enhanced the germination percentage at all doses except at 3.5g kg⁻¹. Maximum germination percentage was with Benlate followed by Thiram and Thiophanate methyl at 3g kg⁻¹. At highest dose of 3.5 g kg⁻¹ GP was significantly lowered for all the fungicides. Fosetyl aluminium and Dimethomorph at 3g kg⁻¹ were statistically at par with the control treatment (Fig. 3).

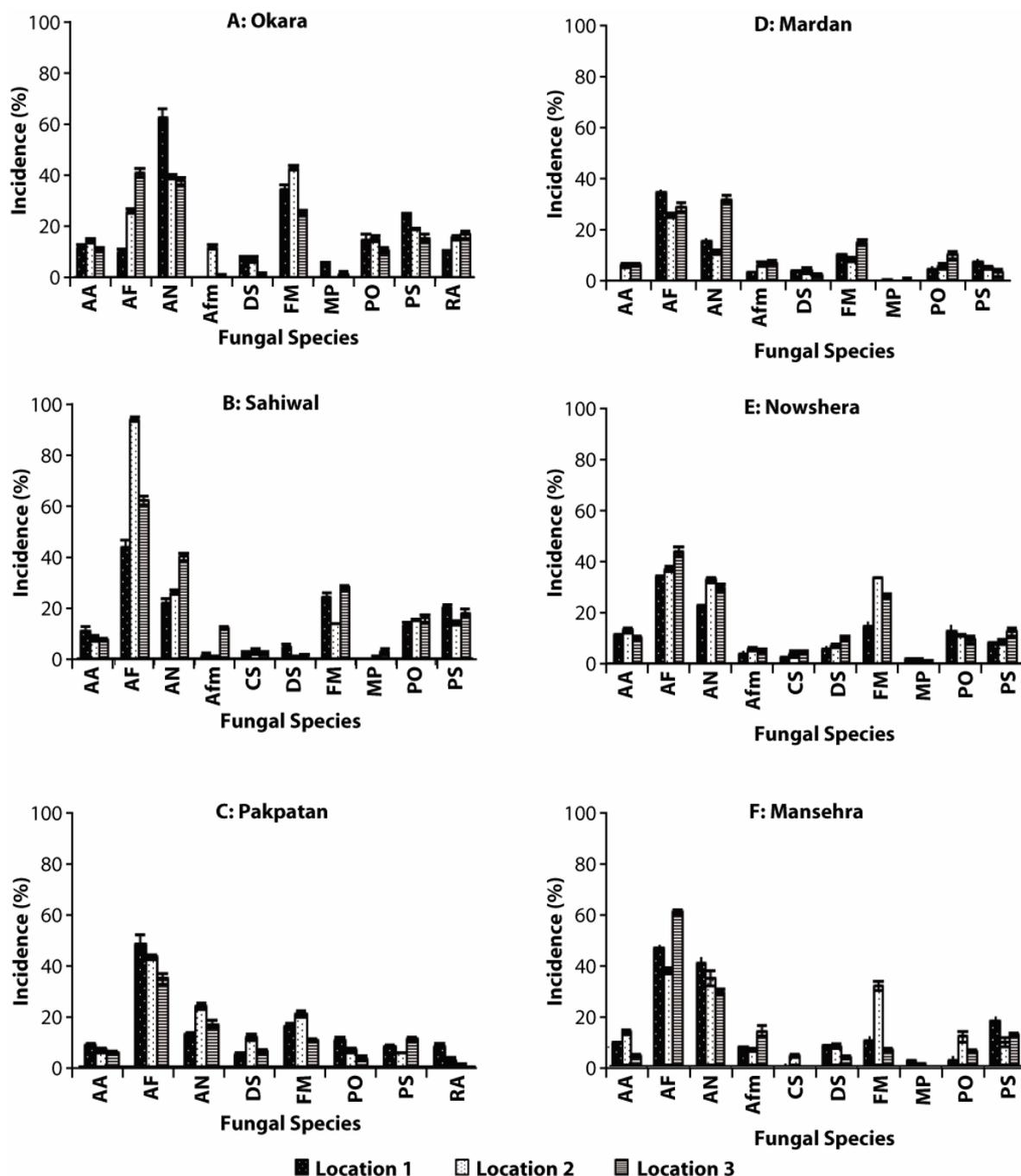


Fig. 1A-F. Fungal incidence percentage for stored maize seed from different maize growing areas of Punjab and Khyber-Paktoonkhwa (Pakistan). AA: *Alternaria alternata*; AF: *Aspergillus flavus*; AN: *Aspergillus niger*; Afm: *Aspergillus fumigatus*; CS: *Cladosporium* sp; DS: *Dreschlera* sp; FM: *Fusarium moniliforme*; MP: *Macrophomina phaseolina*; PO: *Penicillium oxalicum*; PS: *Penicillium* sp.

Present findings for chemical control are in conformity with some previous investigations on various crop seeds (Bhutta *et al.*, 2001; Pathan *et al.*, 2004; Bhutta *et al.*, 2004; Siddiqui & Zaman, 2004). However the results were partially contrary to earlier findings of Javaid *et al.*, (2006) and Sitara & Akhtar (2007) and highly contrasting to Dey *et al.*, (1988), Bharath *et al.*, (2005) and Govender *et al.*, (2008) who have recommended some other fungicides for seed treatment. The variation among the above-said studies may be attributed to limited

scale studies by other researchers excluding Benlate and Thiram as well as variability in seed and fungal spectrum. The later-mentioned phenomenon was also observed in present investigations as varied species response against fungicides was evidenced. A positive effect on seed germination has been observed with different fungicides with optimized doses in the present study. A similar effect has been reported by Bhutta *et al.*, (2001); Siddiqui & Zaman (2004) and Javaid *et al.*, (2006).

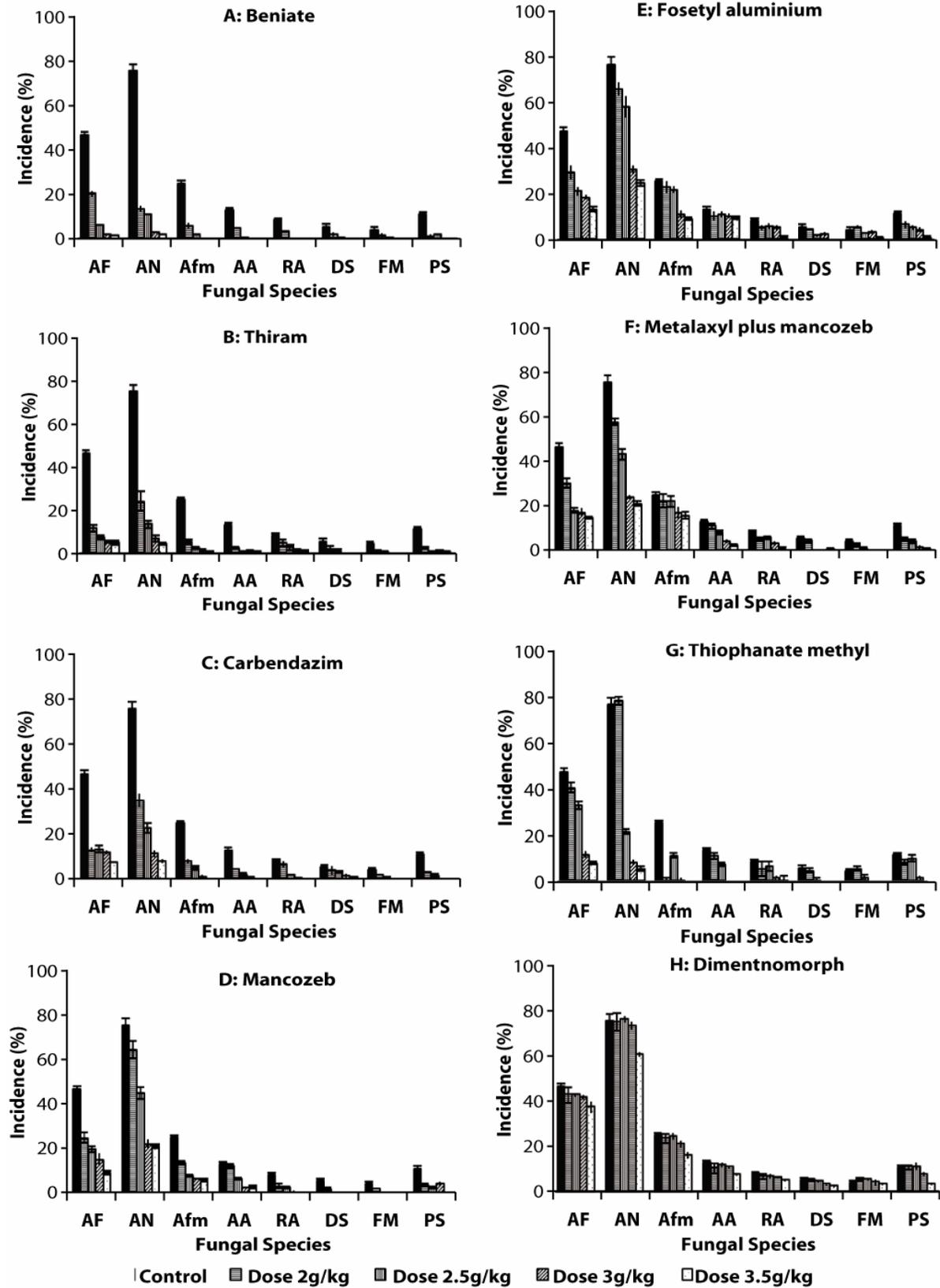


Fig. 2A-H. Fungal incidence percentage (IP) with different fungicides. AA: *Alternaria alternata*; AF: *Aspergillus flavus*; AN: *Aspergillus niger*; Afm: *Aspergillus fumigatus*; DS: *Dreschlera* sp.; FM: *Fusarium moniliforme*; PS: *Penicillium* sp.; RA: *Rhizopus arrhizus*

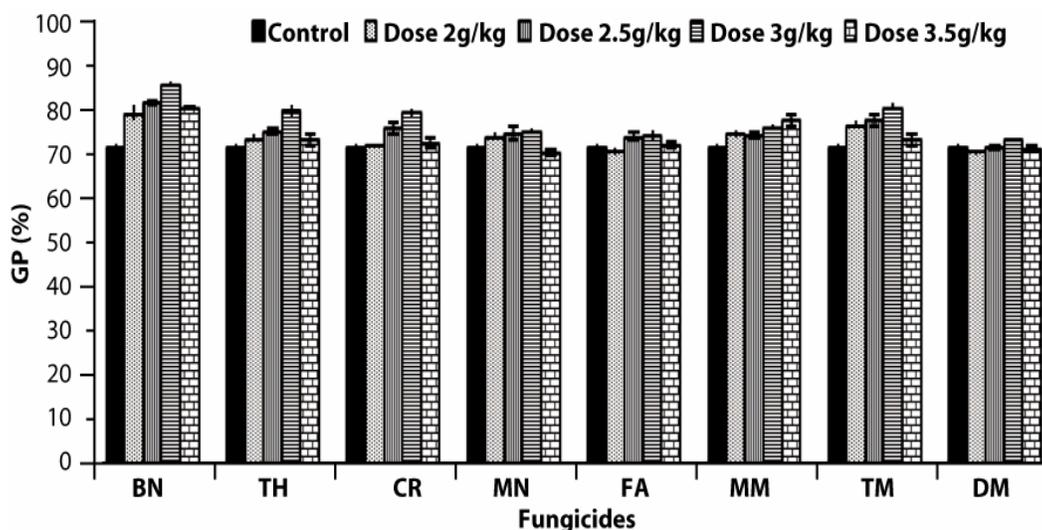


Fig. 3. Germination percentage (GP) under different fungicidal treatments.

BN: Benlate; TH: Thiram; CB: Carbendazim; MN: Mancozeb; FA: Fosetyl aluminium; MM: Metalaxyl plus mancozeb; TM: Thiophanate methyl; DM: Dimethomorph

In the present study, maize seed mycoflora was found to be an extensive and threatening problem in maize growing areas of Pakistan. *A. flavus*, *A. niger* and *Fusarium moniliforme* have been recorded as the most prevalent fungal species in all the samples. Maximum number of fungal species i.e. 10 was observed from Okara, Sahiwal and Nowshera districts. Benlate, Thiophanate Methyl and Thiram provided the maximum fungal control at 3 and 3.5 g kg⁻¹. Benlate being equally effective at 3 g kg⁻¹ and also having positive effect for germination percentage was found to be most suitable fungicide for maize seed treatment. It is therefore, recommended to use the suitable fungicide with appropriate dosage to arrest the fungi along with enhancement of germination.

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