



RESEARCH PAPER

Efficacy of fungicides, phytoextracts and bioagent for the management of seed mycoflora of chickpea

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Abstract : A laboratory experiment was conducted to evaluate the efficacy of fungicides, phytoextracts and bioagent for managing the seed-borne fungi associated with chickpea. Results revealed that all the treatments were effective in reducing seed mycoflora load over control. However, seed treatment with carboxin + thiram at 0.3 per cent was found superior as it witnessed minimum number of fungal species (2) and minimum seed mycoflora load (2.75%) followed by mancozeb at 0.3 per cent and carbendazim at 0.25 per cent. Carboxin + thiram completely inhibited the growth of *Aspergillus flavus*, *Rhizopus* sp., *Botrytis* sp. and *Curvularia lunata*. Seed treatment with *Trichoderma viride* found effective in reducing seed mycoflora compared to control. Among the phytoextracts, the least per cent seed mycoflora (33.55%) was observed in *Neem* (*Azadirachta indica* L.) leaf extract whereas datura leaf extract and garlic clove extract significantly reduced the seed mycoflora.

Key Words : Fungicides, Phytoextracts, Bioagent, Management, Seed mycoflora, Chickpea

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INTRODUCTION

Chickpea (*Cicer arietinum* L.), is the third most important pulse crop in the world, also known as “King of pulses”. Its significant contribution towards nutritional security as well as soil fertility management accomplished its extensive cultivation in more than 50 countries occupying an area of 13.7 million hectares with the production of 14.2 million tons. India is the world’s leading producer and consumer of chickpea. The other major chickpea producing countries includes Turkey, Russia, Myanmar, Pakistan, Ethiopia, USA, Australia, Canada and Mexico (Anonymous, 2019a).

In India, it is the prime cool season pulse crop cultivated in an area of 10.56 million ha with an annual production of 11.38 million tonnes and productivity of 1078 kg per ha. Nearly 90% of the crop is cultivated in rainfed mostly on receding soil moisture and on marginal lands. Madhya Pradesh stands first in production followed by Maharashtra, Rajasthan, Karnataka and Andhra Pradesh (Anonymous, 2019b).

Pathogens are the major constraints in economic production of chickpea among them, seed-borne pathogens have a lion’s share. They are responsible for discoloration and deterioration of seeds, reduction in

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viability, germination and vigour and produce abnormal seedlings. Infected seeds serve as a vehicle for transmitting plant pathogens into new areas resulting in a serious disease outbreak hampering the plant growth and productivity. It is also reported that some of these fungi produce aflatoxins and induce carcinogenesis, mutagenesis and teratogenesis (Pereyra *et al.*, 2008). Therefore, controlling seed-borne fungi is pivotal and the harmful effects can be mitigated through integrated approaches.

Seed treatment for plant disease management has been coined the “painless approach” for farmers. It is highly relevant to developing countries like India, where the majority of farmers are small and marginal, unable to afford the high costs of spraying and dusting. Seed treatment with fungicides can dramatically reduce disease and as a result, increase genetic potential and yield. Biological agents such as *Trichoderma* sp., *Bacillus* sp. and *Pseudomonas* sp. were found to be effective in controlling a wide range of seed-borne fungi with no risk of resistance being produced. Plant-derived natural products are another source of potential new pesticides. Plant extracts and essential oils have antifungal properties against a wide variety of fungi (Abd-Alla *et al.*, 2001).

MATERIAL AND METHODS

Laboratory experiment was conducted at the Department of Plant Pathology, BACA, AAU, Anand to evaluate the efficacy of fungicides, phytoextracts and bioagent for managing the seed-borne fungi associated with chickpea. Three fungicides (carbendazim, carboxin + thiram and mancozeb), three phytoextracts (datura, neem and garlic) and a bioagent (*Trichoderma viride*) were evaluated as seed treatment at their respective concentrations by Agar plate method. Completely Randomized Design (CRD) was employed with 8 treatments and 4 repetitions and each repetition having 100 seeds.

Treatments details :

T₁: Carbendazim 50 WP @ 2.5 g /kg seeds

T₂: Carboxin 37.5+Thiram 37.5 WS @ 3 g/kg seeds

T₃: Mancozeb 75 WP @ 3 g /kg seeds

T₄: Datura (*Datura stramonium* L.) leaf extract @ 10%

T₅: *Neem* (*Azadirachta indica* L.) leaf extract @ 10%

T₆: Garlic (*Allium sativum* L.) clove extract @ 10%

T₇: *Trichoderma viride* @ 2×10^8 cfu/ml

T₈: Untreated check (Control)

Treated seeds were kept in agar plate at 10 seeds/plate and the plates were incubated at 25 ± 2 °C for 7 days giving 12/12 alternate cycle of light and darkness of Near Ultra Violet (NUV). Seed mycoflora load with respect to number of colonies and types of fungi was recorded.

Preparation of phytoextracts :

Fresh and healthy leaves of *Neem* and datura was collected and rinsed thoroughly under running tap water. These leaves were chopped into small pieces and macerated in sterilized distilled water (1:1 w/v basis) by blender. Likewise, healthy cloves of garlic, after removing the outer layer were macerated in sterilized distilled water by blender. The crude extract obtained from each plant was filtered through a single layer of sterilised muslin cloth and the filtered extracts was regarded as stock (100%) solutions. Stock solutions were further diluted to the desired concentration (10%) by adding the appropriate amount of water and then used for pre-soaking for 5 minutes.

Culturing of *Trichoderma viride* :

The pure culture of *T. viride* (TNAU isolate) available in the Department of Plant Pathology, AAU, Anand was cultured on PDA for one week at 28 ± 2 °C and the spore suspension of the fungi was prepared in distilled water so as to obtain 2×10^8 cfu/ml for seed treatment.

RESULTS AND DISCUSSION

Results from the present investigation revealed the significant differences in per cent seeds showing mycoflora growth (Table 1). None of the treatments gave absolute control of all fungi. However, carboxin + thiram @ 0.3 per cent (T₂) documented minimum number of fungal species (2) and minimum seed mycoflora load (2.75%) followed by mancozeb @ 0.3 per cent (T₃) and carbendazim @ 0.25 per cent (T₁). Better performance of fungicide carboxin + thiram can be attributed to its broad spectrum and dual action (systemic and contact) properties.

Similar results regarding effectiveness of various fungicides were reported earlier by Nikam *et al.* (2007) who recorded that seed treatment of chickpea with thiram

(0.15%) + carbendazim (0.1%) effectively inhibited the growth (90%) of *F. oxysporum* f. sp. *cicerii* *in vitro* with colony diameter (9 mm) and found superior to rest of fungicide treatments. Dolas *et al.* (2018) reported that mung bean seeds treated with carbendazim @ 0.2 % recorded 8.4 per cent seed mycoflora, compared to 51.2 percent in the control treatment. This fungicidal treatment reduced seed mycoflora by 83.59% compared to the control.

Aspergillus flavus, *Rhizopus* sp., *Botrytis* sp. and *Curvularia lunata* did not grow on seeds treated with carboxin + thiram (T₂) whereas Mancozeb @ 0.3 per cent (T₃) completely inhibited the growth of *Rhizopus* sp. and *Curvularia lunata*. With respect to per cent seeds showing mycoflora, control (T₈) recorded significantly highest per cent mycoflora (91.12), followed by garlic clove extract treatment (T₆) and datura leaf extract treatment (T₄), which showed seed mycoflora of 61.12 and 44.87 per cent, respectively.

Among the phytoextracts, the least per cent seed mycoflora was observed in *Neem* (*Azadirachta indica* L.) leaf extract (33.55%) followed by datura (*Datura stramonium* L.) leaf extract (44.87%) whereas garlic (*Allium sativum* L.) clove extract recorded significantly high mycoflora load (61.12%).

These results are in accordance with the findings

of Mahal (2014) who reported that the incidence of *Aspergillus flavus* in chickpea seeds was successfully controlled with *Allium sativum* and *A. indica* extracts whereas *A. indica* and *Zingiber officinale* absolutely controlled *Penicillium* sp. Seed mycoflora of mungbean were successfully controlled with *A. sativum* extract while *Curvularia* sp. and *F. oxysporum* were absolutely controlled with *Z. officinale* extract.

The results revealed that seed treatment with *Trichoderma viride* (2×10⁸ cfu/ml) found effective in reducing seed mycoflora in chickpea as compared to control. Similar results have been shown by Zanjare *et al.* (2016) who revealed that seed treatment of chickpea with *T. viride* was effective in reducing seed mycoflora *i.e.* *Fusarium oxysporum*, *Curvularia lunata*, *Alternaria alternata*, *Aspergillus niger* and *A. flavus* by 45.0, 37.8, 56.5, 63.0 and 30.4 per cent, respectively over untreated control.

These findings also confirmed that *Trichoderma viride* (19.87%), *Neem* leaf extract (33.55%), datura leaf extract (44.87%) and garlic clove extract (61.12%) was effective in reducing seed mycoflora compared to control (91.12%) and hence proved its potentiality as an eco-friendly approach for the management of seed mycoflora.

Table 1: *In vitro* management of seed mycoflora of chickpea

Tr. No.	Treatments	Conc. (%) /Dose	Seed mycoflora load (%)							Total (%)
			<i>A. niger</i>	<i>A. flavus</i>	<i>F. oxysporum</i> f. sp. <i>ciceris</i>	<i>Rhizopus</i> sp.	<i>Botrytis</i> sp.	<i>Curvularia lunata</i>	Total fungal species	
T ₁	Carben dazim 50 WP	0.25	2.50	2.25	2.75	0.00	1.25	0.75	5	9.50
T ₂	Carboxin 37.5 + Thiram 37.5 WS	0.30	1.25	0.00	1.50	0.00	0.00	0.00	2	2.75
T ₃	Mancozeb 75 WP	0.30	2.25	1.75	2.25	0.00	1.00	0.00	4	7.25
T ₄	Datura (<i>Datura stramonium</i> L.) leaf extract	10.00	11.75	7.50	8.25	7.75	6.75	2.87	6	44.87
T ₅	Neem (<i>Azadirachta indica</i> L.) leaf extract	10.00	8.75	5.75	5.75	6.25	6.00	1.00	6	33.50
T ₆	Garlic (<i>Allium sativum</i> L.) clove extract	10.00	15.25	12.00	11.50	9.25	8.25	4.87	6	61.12
T ₇	<i>Trichoderma viride</i>	2×10 ⁸ cfu/ml	7.50	3.25	5.50	0.00	2.75	0.87	5	19.87
T ₈	Untreated check (Control)	-	21.75	16.75	18.50	13.50	11.50	9.12	6	91.12
	S.E. ±		0.36	0.27	0.27	0.18	0.20	0.10		
	C.D. (P=0.05)		1.05	0.77	0.79	0.54	0.60	0.30		
	C. V. %		8.13	8.61	7.72	8.01	8.71	8.37		



T₁ : Carbendazim



T₂ : Carboxin + Thiram



T₃ : Mancozeb



T₄ : Datura leaf extract



T₅ : *Neem* leaf extract



T₆ : Garlic clove extract



T₇ : *Trichoderma viridae*



T₈ : Control

Fig. 1 : Management of seed mycoflora

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