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RESEARCH ARTICLE :

In vitro efficacy of various seed dresser on seed mycoflora of safflower

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SUMMARY : Seeds play vital role for the healthy production of crop and they are known to carry pathogens which cause poor seed health. Safflower (*Carthamus tinctorius* L.) occupies prominent place in the agricultural wealth and economy of the country. Safflower being rich source of proteins and edible oil has focused attention of farmers. Studies on seed mycoflora have greatly increased in the recent past in view of their importance as diseases carriers, deteriorating agents and also as toxin produces. *In vitro* efficacy of various seed dresser fungicides (systemic and non systemic) on the seed mycoflora improving per cent seed germination, seedling vigour, per cent seedling mortality and per cent infection. It was observed that maximum seed infection with *A. carthami* was recorded in treatment Thiram+ Carbendazim (10.50%) and minimum seed infection with *M. phaseolina* Thiram + Mancozeb (0.00%), respectively. Effect of nine seed dresser fungicides on the per cent seedling vigour during the studies it was observed that fungicides improving the per cent seedling vigour and reducing per cent seedling mortality by rolled towel paper method. Maximum per cent of seedling vigour observed in Thirum + Mancozeb (16%) and minimum per cent of seedling vigour observed in treatment Thiram+ Carbendazim (13%). In per cent seedling mortality the there was no mortality observed in 24 and 48 hrs. The lowest per cent seedling mortality was observed in seed treatment Thirum + Mancozeb (5.5%) and maximum per cent in the Thirum + Carbendazim (10.9%) at 72 hrs.

KEY WORDS:
Seed mycoflora, Seed dresser, *Alternaria carthami, Fusarium carthami, Macrophomina phaseolina*, Safflower

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BACKGROUND AND OBJECTIVES

Economically India occupies first position in area of safflower followed by USA. Maharashtra rank first in area and production accounting as 72 per cent and 69 per cent, respectively. During year 2014-15 the area and production safflower in India is 2.96 M/ ha and 1.80 MT, respectively, with the productivity of 609 kg/ ha. In Maharashtra area and production of safflower is 1.91 M/ ha and 1.44 MT, respectively, with the productivity of 594 kg/ ha (Anonymous, 2014).

Safflower is also affected by many biotic and abiotic stresses. Of the biotic agents, fungi cause major diseases, followed by bacteria, viruses and nematodes. Major safflower diseases caused by fungi are; Leaf spot / blight (*Alternaria carthami*),Wilt (*Fusarium*

oxysporum f.sp. *carthami*), Root rot (*Rhizoctinia bataticola*), Powdery mildew (*Erysiphe cichoracearum*) and Anthracnose (*Colletotrichum capsici*), bacterial Leaf blight/spot (*Pseudomonas*), viral diseases such as Mosaic (*Cucumber mosaic virus*), Necrosis (Tobacco streak virus) and root knot (*Meliodogyne hapla*) nematode. Among these diseases leaf spot / blight caused by *Alternaria carthami* (Chowdhury) is wide spread and have continued to be the major constraints in the production and productivity of safflower all over the country in general as well as particularly in the state of Maharashtra. The disease (*A. carthami*) has been reported to cause yield losses to the tone of 25 to 60 per cent all over India (Singh and Prasad, 2005) and 20 to 80 per cent in Maharashtra state (Anonymous, 2006 and 2010), along with drastic reduction in seed size, seed volume, seed test weight as well as per cent oil content. Under severe infection, disease has been reported to cause 50 per cent loss in seed yield (Indi *et al*., 1986).

So keeping in view the economic importance of safflower and serious nature of the disease, the present studies were planned and conducted in year 2015-16 at Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani

RESOURCES AND METHODS

For *in vitro* evaluation of seed dressers lot of hundred seeds were collected and treated as per the recommended dose with the different (seed dressers) fungicides and observations were recorded at 24hrs, 48hrs, 72hrs, 120hrs, 144hrs and 168hrs a by rolled towel paper method for per cent seedling vigour and per cent seedling mortality (as described below) and the per cent seed mycoflora were recorded at 7 DAT by blotter paper method (as described below). For each seed treatment and interval three replications were maintained along with untreated control.

Experimental details :

Design : CRD (Completely Randomized Design)
Replications : Three
Treatments : Ten

Year of Experiment: 2015-16 :

Treatments	Common name	Formulation
T_1	Thiram	75WP
T_2	Cardendazim	50WP
T_3	Carboxin	75WP
T_4	Mancozeb	75WP
T_5	Thiram + Carbendazim	-
T_6	Thiram + Carboxin	-
T_7	Thiram + Mancozeb	-
T_8	Carbendazim+ Carboxin	-
T_9	Carbendazim + Mancozeb	75WP
T_{10}	Control	

Detection of per cent seed mycoflora:

Blotter paper method:

Three layers of blotter paper size equivalent of petridish were soaked in sterile water and kept in the petridish. Seeds of safflower were selected for detection of internal seed-borne fungi. These seeds were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 1 to 2 minute, and wash in three changes of sterile water. Ten seeds were placed equidistantly on three layers of moist blotter paper in Petridish. The external seed-borne fungi were observed by placing seeds directly on the three layers of moist blotters in Petridish. All petridishes were incubated at 26° C+ 2°C and exposed to ultraviolet light and dark for 7 days. Sterilized distilled water added to moisten the blotter paper as and when required. Seeds and seedlings were examined after seven days using stereo binocular microscope for the individual seed mycoflora.

Detection of per cent seedling vigour and per cent seedling mortality :

Rolled towel method:

Seeds were surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 1 to 2 minute and subjected to subsequent three washing of sterile water. Hundred seeds were placed on moist towel paper and covered with polythene paper and rolled carefully avoiding disturbance of the seeds. The rolled towel papers were kept in slanting position along the wall of laboratory tables and incubated at 26 ± 2° C for 7 days. The seeds and seedlings were examined with stereo binocular microscope and the observations on seedling vigour and seedling mortality were recorded at 24 hrs interval upto 168 hrs for individual seed mycoflora.

The seedling vigour Index was calculated by formula suggested by Baki and Anderson (1973).

Seedling vigour Index (%) = (Root + Shoot length in cm) x Germination %

Seedling mortality was calculated by formula

$$\% \text{ seedling mortality } Z \frac{\text{No. of seedlings died}}{\text{Total no. of seedlings}} \times 100$$

OBSERVATIONS AND ANALYSIS

Nine seed dressing fungicides as per the recommended dose [*viz*., Thiram, Carbendazim, Carboxin, Mancozeb, Thiram + Carbendazim, Thiram+ Carboxin, Thiram+ Mancozeb, Carbendazim+ Carboxin and Carbendazim+ Mancozeb were evaluated *in vitro* against safflower seed mycoflora by rolled towel paper method effect of these fungicides in per cent seed mycoflora were recorded after 7 days of incubation and results obtained are presented in (Table 1) and Plate 1.

Results (Table 1) revealed that among these seed dresser fungicides, the lowest seed infection with *Macrophomina phaseolina* was observed in seed treatment Thiram + Carboxin, Thiram+ Mancozeb and Carbendazim+Mancozeb (0.00%) and maximum per cent seed infection with *Alternaria carthami* was observed in treatment Thiram + Carbendazim (10.50 %).

The maximum per cent seed infection with *Alternaria carthami* was observed in seed treatment Thiram+Carbendazim (10.50 %) whereas, the lowest per cent seed infection was observed in seed treatment Thiram + Mancozeb (6.66%) which was significantly superior over all the treatments.

The maximum per cent of seed infection with *Fusarium oxysporum* f. sp. *carthami* was observed in seed treatment Thiram + Carbendazim (8.00 %). However, the lowest seed infection with *Fusarium oxysporum* f. sp. *carthami* was observed in seed treatment Thiram + Mancozeb (3.33%) which was significantly superior over all the treatments.

The maximum per cent of seed infection with *Macrophomina phaseolina* was observed in seed treatment Thiram+Carbendazim (3.64 %) whereas, the lowest seed infection with *Macrophomina phaseolina* was observed in seed treatment Thiram+ Mancozeb (0.00 %) which was significantly superior over all the treatments.

Result of the present studies on efficacy of various seed dresser on seed mycoflora are in consonance with those reported earlier by several workers (Thomas 1950, Pandganur and Anil Kumar, 1976, Siddaramaiah *et al.*, 1980).

In vitro efficacy of various seed dresser on seedling vigour :

Nine seed dresser fungicides as per the recommended dose *viz*., Thiram, Carbendazim, Carboxin, Mancozeb, Thiram + Carbendazim, Thiram+ Carboxin, Thiram+ Mancozeb, Carbendazim+ Carboxin and Carbendazim+ Mancozeb were evaluated *in vitro* for per cent seedling vigour of safflower by rolled towel paper method. Effect of these fungicides on per cent seedling vigour were recorded at 24 hrs interval upto 168 hrs incubation and results obtained are presented in (Table 2) and Plate 2.

Result (Table 2) revealed that among all the treatments there was no seedling vigour observed at 24

Table 1 : *In vitro* efficacy of various seed dresser on seed mycoflora

Tr. No.	Treatments	Per cent seed mycoflora infection after 7 day incubation		
		Alternaria carthami	*Fusarium carthami*	*Macrophomina phaseolina*
T_1	Thiram	9.33 (17.79)	6.00 (14.18)	1.50 (7.03)
T_2	Carbendazim	10.00 (18.43)	5.50 (13.56)	2.31 (8.74)
T_3	Carboxin	8.33 (16.78)	5.00 (12.92)	1.00 (5.74)
T_4	Mancozeb	8.33 (16.78)	5.33 (13.35)	1.00 (5.74)
T_5	Thiram + Carbendazim	10.50 (18.91)	8.00 (16.43)	3.64 (11.00)
T_6	Thiram + Carboxin	7.66 (16.07)	4.33 (12.01)	0.00 (0.00)
T_7	Thiram + Mancozeb	6.66 (14.96)	3.33 (10.51)	0.00 (0.00)
T_8	Carbendazim+Carboxin	8.00 (16.43)	4.66 (12.47)	0.00 (0.00)
T_9	Carbendazim+Mancozeb	10.50 (18.91)	7.00 (15.34)	3.00 (9.97)
T_{10}	Control	42.54 (40.71)	13.54 (21.59)	9.68 (18.13)
	S.E. $\pm$	0.52	0.37	0.25
	C.D. (P=0.01)	1.72	1.20	0.82

Figures in parentheses are angular transformed values

Table 2 : *In vitro* efficacy of various seed dresser on seedling vigour

Tr. No.	Treatments	Per cent seedling vigour at 24hrs to 168 hrs incubation						
		24 hrs	48 Hrs	72 hrs	96 hrs	120 hrs	144 Hrs	168 hrs
T_1	Thiram	0.00	13.5	55.0	271.4	428.4	481.6	533.4
T_2	Carbendazim	0.00	13.1	54.7	265.8	430.4	472.6	527.1
T_3	Carboxin	0.00	14.0	58.2	275.6	438.1	479.6	531.6
T_4	Mancozeb	0.00	14.2	61.5	278.6	435.8	490.7	540.1
T_5	Thiram + Carbendazim	0.00	13.0	52.1	266.4	430.5	461.7	502.1
T_6	Thiram + Carboxin	0.00	15.8	65.4	284.7	440.8	502.5	552.4
T_7	Thiram + Mancozeb	0.00	16.0	69.7	290.4	447.9	518.9	561.5
T_8	Carbendazim+Carboxin	0.00	14.6	62.4	280.7	439.4	496.7	541.2
T_9	Carbendazim+Mancozeb	0.00	13.5	48.4	261.5	426.4	451.2	497.5
T_{10}	Control	0.00	9.6	41.5	251.2	321.5	364.1	397.5
	S.E. $\pm$	0.00	0.41	0.40	0.44	0.48	0.41	0.40
	C.D. (P=0.01)	0.00	1.53	1.30	1.45	1.57	1.35	1.32

Table 3 : *In vitro* efficacy of various seed dresser on per cent seedling mortality

Tr. No.	Treatments	Per cent seedling mortality at 24 to 168 hrs incubation						
		24hrs	48hrs	72hrs	96hrs	120hrs	144hrs	168hrs
T_1	Thiram	0.00 (0.00)	0.00 (0.00)	9.6 (18.05)	10.5 (18.91)	11.9 (20.18)	14.8 (22.63)	17.6 (24.80)
T_2	Carbendazim	0.00 (0.00)	0.00 (0.00)	9.4 (17.85)	10.8 (19.19)	13.4 (21.47)	15.9 (23.50)	18.6 (25.55)
T_3	Carboxin	0.00 (0.00)	0.00 (0.00)	8.4 (16.85)	9.1 (17.56)	12.4 (20.62)	12.4 (20.62)	15.8 (23.42)
T_4	Mancozeb	0.00 (0.00)	0.00 (0.00)	7.5 (15.89)	8.7 (17.16)	11.6 (19.91)	13.5 (21.56)	16.4 (23.89)
T_5	Thiram + Carbendazim	0.00 (0.00)	5.3 (13.3)	10.9 (19.28)	12.5 (20.70)	14.5 (22.38)	17.1 (24.43)	22.5 (28.32)
T_6	Thiram + Carboxin	0.00 (0.00)	0.00 (0.00)	7.8 (16.22)	8.3 (16.74)	10.5 (18.91)	11 (19.37)	13.5 (21.56)
T_7	Thiram + Mancozeb	0.00 (0.00)	0.00 (0.00)	5.5 (13.56)	8.2 (16.64)	8.8 (17.26)	9.8 (18.24)	11.2 (19.55)
T_8	Carbendazim+ Carboxin	0.00 (0.00)	0.00 (0.00)	8.1 (16.54)	8.8 (17.26)	10.9 (19.28)	12.5 (20.70)	15.00 (22.79)
T_9	Carbendazim+Mancozeb	0.00 (0.00)	0.00 (0.00)	10.5 (18.91)	11.8 (20.09)	14.6 (22.46)	16.9 (24.27)	20.1 (26.64)
T_{10}	Control	0.00 (0.00)	6.2 (14.42)	19.2 (25.99)	28.4 (32.20)	39.7 (39.06)	49.5 (44.71)	64.1 (53.19)
	S.E. $\pm$	0.00	0.26	0.48	0.50	0.48	0.43	0.45
	C.D. (P=0.01)	0.00	0.86	1.56	1.66	1.57	1.40	1.47

Figures in parentheses are angular transformed values

hrs due to the germination. At 48 hrs. the lowest per cent seedling vigour was observed in seed treatment Thiram + Carbendazim (13%) whereas, the maximum per cent seedling vigour observed in treatment Thiram+ Mancozeb (16%), which was significantly superior over all the treatments.

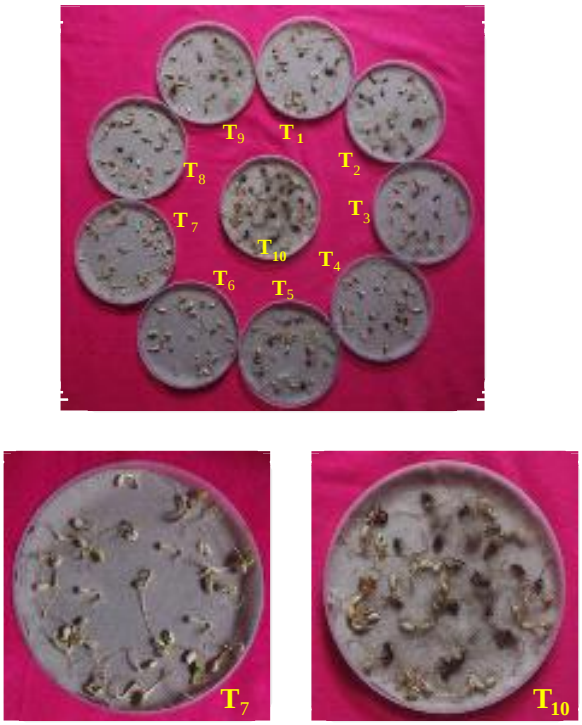
All the seed dresser fungicides tested exhibited similar trends of per cent seedling vigour at 72 hrs, 96 hrs, 120 hrs, 144 hrs and 168 hrs as that of 48 hrs observations.

Result of the present studies on effect of seed dresser on seedling vigour are in consonance with those reported earlier by several workers (Suzer and Schneiter, 1994 and Siddaramaiah and Hegde, 1983).

In vitro efficacy of seed dresser on per cent seedling mortality :

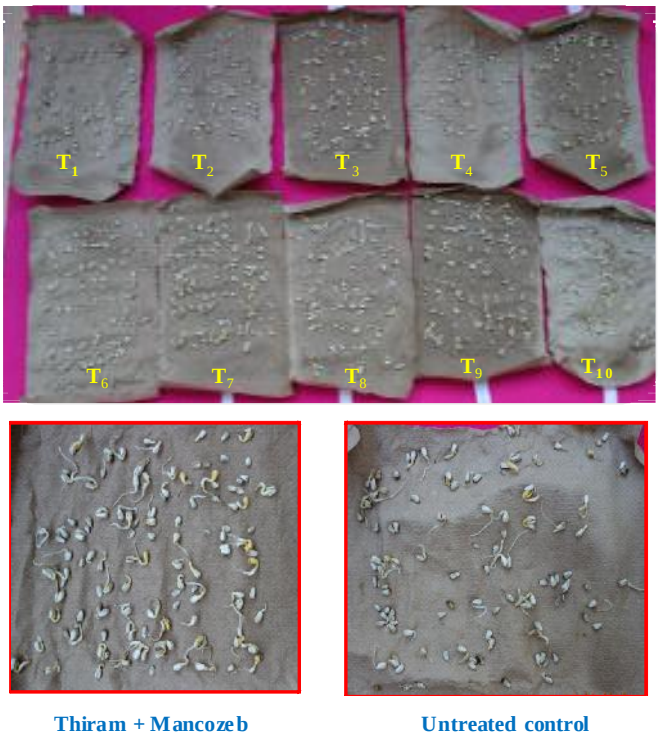
Nine seed dresser fungicides *viz*., Thiram, Carbendazim, Carboxin, Mancozeb, Thiram + Carbendazim, Thiram+ Carboxin, Thiram+ Mancozeb, Carbendazim+ Carboxin and Carbendazim+ Mancozeb were evaluated *in vitro* for per cent seedling mortality by rolled towel paper method. Effects of these fungicides on per cent seedling vigour were recorded at 24 hrs interval upto 168 hrs of incubation and results obtained are presented in Table 3 and Plate 2.

Among all the seed treatments; there was no seedling mortality observed at 24hrs. At 48 hrs maximum per cent of seedling mortality was observed in treatment Thiram+Carbendazim (5.3 %) whereas, all treatments



T_1	Thiram	T_6	Thiram + Carboxin
T_2	Carbendazim	T_7	Thiram + Mancozeb
T_3	Carboxin	T_8	Carbendazim+ Carboxin
T_4	Mancozeb	T_9	Carbendazim+ Mancozeb
T_5	Thiram + Carbendazim	T_{10}	Control

Plate 1 : *In vitro* efficacy of seed dressers on per cent seed mycoflora



T_1	Thiram	T_6	Thiram + Carboxin
T_2	Carbendazim	T_7	Thiram + Mancozeb
T_3	Carboxin	T_8	Carbendazim+ Carboxin
T_4	Mancozeb	T_9	Carbendazim+ Mancozeb
T_5	Thiram + Carbendazim	T_{10}	Control

Plate 2 : *In vitro* efficacy of various seed dressers on per cent seedling vigour and per cent seedling mortality

showed no mortality.

At 72 hrs maximum per cent of seedling mortality with *Alternaria carthami* was observed in treatment Thiram + Carbendazim (10.9%) and lowest per cent seedling mortality was observed in seed treatment Thiram+ Mancozeb (5.5%), which was significantly superior over all the treatments.

All the seed dresser fungicides tested exhibited similar trends of per cent seedling mortality at 96 hrs, 120 hrs, 144 hrs and 168 hrs as that of 72 hrs observations.

Result of the present study on effect of seed dresser against per cent seedling mortality of *A. carthami* are in consonance with those reported earlier by several workers (Padganur and Anil Kumar, 1976; Irwin and Jackson, 1976; Chakrabarti and Basuchaudhari, 1980 and Charjan and Tarar, 1991).

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