The Bioscan 9(3): 1221-1224, 2014 (Supplement on Plant Pathology)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFICACY OF FUNGICIDAL AND BIOCIDAL (PLANT EXTRACTS AND BIO AGENTS) SEED TREATMENT IN MANAGEMENT OF SEED BORNE MYCOFLORA OF FENNEL

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KEYWORDS
Aspergillus niger
Bioagents
Fungicides
Plant extracts

Received on :
24.05.2014

Accepted on :
20.08.2014

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ABSTRACT
Among fungicides used as seed treatment, *in vitro* Captan proved to be most effective against *Aspergillusniger* followed by Thiram, Bavistin in improving seed germination and vigourindex by reducing pre and post emergence mortality and number of seedlings showing symptoms. Similarly, among plant extract and bioagents used, garlic extracts followed by neem extracts and *Trichoderma harzianum* followed by *Trichodermaviride*proved to be most effective in improving seed germination and vigour index by reducing pre and post emergence mortality and number of seedlings showing symptoms.

INTRODUCTION

In India, fennel is mostly grown in north India and the important producing states are Gujarat, Rajasthan, Madhya Pradesh, Haryana and Uttar Pradesh (Sastry *et al.*, 2009).It is good source of compounds with medicinal value such as anti-oxidant, antimicrobial, anti-inflammatory, diuretic etc. (Rahimi and Ardekani, 2013; Annies Remedy, 2014). Fennel seeds are known for their fragrant odour, pleasant aromatic taste, high nutritive value, stimulating and carminative properties widely used in our daily food as soups, pickles, meal dishes, sauces, pastries, confectionaries and it is also used as important ingredients of allopathic as well as ayurvedic medicine. Besides this, fennel also plays a significant role in our national economy (Vanangamudi and Natrajan, 2008).The reproduction and growth of various fungi is inhibited by many fungicides (Chaudhary and Chaudhari, 2013). Spores of the fungi are one of most important part used in the identification and classification of the organism (Jambhulkar *et al.*, 2012).Among several factors which reduced the productivity of fennel seeds quantitatively and qualitatively the use of self-stored saved seeds invaded by different field and storage fungi during their course of development on the plants, handling and processing and also during their storage.

MATERIALS AND METHODS

Apparently healthy surface sterilized seeds from seed samples of fennel were artificially contaminated with pathogenic mycoflora and were treated (dressing/soaking) separately by following fungicides, plant extracts and bioagents.

Leaf extracts of each plant leaves was prepared separately by washing the leaves, chopping and grinding them in a mortar and pestle with the addition of cold water at the ratio of 1:2. The extracts were squeezed through cotton wool and used immediately (Alice, 1984). Seed were soaked for 30 minutes (Jacob and Sivaprakasam, 1994).

Inoculated untreated seeds were used as control. Seeds were planted in Petridishes as described under section 1. Observations were recorded after 20 days instead of seven days on seed germination, pre- and post-emergence mortality and root/ shoot length. Seedling vigour was calculated by the following formula suggested(Abdul Baki and Anderson, 1973).

Vigour Index = Germination (%) x (Root length + Shoot length)

RESULTS AND DISCUSSION

Among various mycoflora isolated from seeds and seedlings showing symptoms, *Aspergillus niger* were most predominantly associated with them. Most of the symptoms of disease was caused by *Aspergillus niger* and observed highly pathogenic in causing pre and post-emergence mortality and reducing vigour index and sowing symptoms. Hence for further studies on management was tested for *Aspergillus niger* only.Fungicides is obvious from the data presented in Table-1 revealed that all the seed treating fungicides tested gave significantly higher percentage of germination ranges from 72.00 to 82.00 per cent against 65.00 per cent in control.

Highest per cent of seed germination (82.00%) was recorded in Captan treated seeds followed by Thiram (76.00%), Bavistin (74.00%), Indofil M-45 (72.00%) and Control (65.00%). Among different fungicides tested, Captan proved to be most effective as less pre- and post-emergence mortality were observed in seeds treated with these fungicides followed by Thiram and Bavistin. All the fungicidal treatments were significantly superior in reducing pre- and post-emergence mortality in comparison to control (12.00%/10.29%). All the fungicidal seed treatments significantly increased the vigour index as compared to control. Maximum vigour index (4674) was observed in Captan treated seed followed by Thiram (3724). The control (untreated/inoculated) seeds showed minimum vigour index (2535) and which was significantly lower than the vigour index observed in seed treated with all fungicides.Seed treatment is the cheapest and easiest method of plant disease control. Seed treatment with fungicides is well known for the control of seed mycoflora (Abou-Heilan, 1984). In the present investigation, it was also observed that seed treatment with different fungicides, increased germination percentage and vigour index on one hand and reduced pre- and post-emergence mortality and number of seedlings showing symptom on the other. Among all the seed dressing fungicides tested, Captan and Thiram were found to be the most effective against *Aspergillus niger in vitro* conditions. Captan gave highest percentage of seed germination and minimum pre- and post-emergence mortality with less number of seedlings showing symptoms followed by Bavistin, Dithane M-45 Vitavax and Captan have been reported to be best seed dresser against seed borne *Aspergillus flavus, Aspergillus niger* and other fungi (Sharma *et al.,* 2000 and Gupta *et al.,* 1989).

The result of plant extract for fungicidal/fungistatic property against seed borne *Aspergillus niger* presented in Table 1 indicated that seed treated with plant extracts of all the plant tested gave significantly higher per cent of seed germination ranges from 67.00 to 74.50 per cent in comparison to control (64.00%). Maximum per cent seed germination was observed in seed treated with plant extract of Garlic (74.50%) followed by Neem (73.75%) and Tulsi (72.50%). It is also evident from the Table 2 that plant extract of garlic proved to most effective as less pre- and post-emergence mortality were observed in seed treated with these two plant extracts followed by neem and tulsi. Satyanashi and doddar extract proved to less effective as they have less pre- emergence mortality (7.50% / 11.00%) and post emergence mortality (9.50%/ 10.30%) in comparison to control (13.00%/ 11.40%), respectively.Maximum vigour index was observed in seed treated with garlic (4097) followed by neem (3848) while it was minimum in control (2304). Statistically significant difference was observed in vigour index of all plant extract treated seeds as compared to control. Seed treatments with different biocides (bioagents and plant extracts) have been reported to be safest in comparison to fungicides. Several biocides have been reported to increase seed germination and vigour index by reducing the pre- and post-emergence mortality in several crops including fennel (Jacob and Sivaprakasam, 1994 and Kaur and Mukhopadhyay, 1992). Amongst seeds soaked in different plant extracts, Garlic leaf extract followed by Neem extract found to be most effective against seed borne *Aspergillus niger* in improving seed germination and vigour index by reducing pre- and post-emergence mortality and number of seedling showing symptoms. Extract of different plants also reported to be effective in inhibiting the radial growth of *Aspergillus flavus* (Jariwala*et al.,* 1996 and Jacob and Sivaprakasam, 1994).

Results of bioagents seed treatments against seed borne *Aspergillus niger* in Table 3 indicated that maximum per cent seed germination (76.00%) was recorded in *Trichoderma harzianum* treated seeds followed by *Trichoderma viride* (74.45.%). *Trichoderma harzianum treated* seeds significantly increased the seed germination while seed treated with FK-14 (69.50%) and FL-18 (68.00%) comparison to control (63.00%).*Trichoderma harzianum* and *Trichoderma viride* bioagents were significantly superior in reducing pre and post emergence mortality over control. Maximum reduction in pre and post emergence mortality was observed in *T. harizianum*

1. Fungicides

S. No.	Trade name	Chemical name	Dose (gm /kg seeds)
1.	Bavistin	Methyl benzimidazolecarbamate	2.0
2.	Captan	N-Trichloromethyl thio-4 cyclohexane -1, 2 dicarboximide	2.0
3.	Thiram	Tetramethylthiouramdisulphide	2.0
4.	Vitavax	5,6 dihydro-2-methyl-1, 4-oxathin-3-carboxgvilide	2.0
5.	Indofil M-45	Manganese ethylene bis-dithiocarbomate plus zinc	2.0
6.	Control	No chemical	-

2. Plant extracts

S. No.	Plant extracts	Botanical name	Dose (Crude extract)
1.	Doddar	*Cuscuta sp.*	@ 10 ml/kg seed
2.	Tulsi	*Ociumum sancium*	@ 10 ml/kg seed
3.	Satyanashi	*Argemone mexicana*	@ 10 ml/kg seed
4.	Garlic	*Allium sativum*	@ 10 ml/kg seed
5.	Neem	*Azadirachta indica*	@ 10 ml/kg seed
6.	Control	(untreated)	-

3. Bioagents

S.No.	Bioagent	Dose (g/kg seed)*
(i)	*Trichoderma harzianum*	4 g
(ii)	*T. viride*	4 g
(iii)	*Pseudomonas fluorescens*	4 g
(iv)	*Pseudomonas putida*	4 g
(v)	*Microbacterium paraoxidans*	4 g
(vi)	Control (untreated)	-

* = 10^8cfu/ml

Table 1: Efficacy of seed dressing, fungicides against *Aspergillus niger* on seed germination, pre- and post emergence mortality and vigour index (*in vitro*)

S. No.	Treatments	Dose(ml/kg seeds)	Per cent Germination	Per cent mortality		Root* elongation (mm)	Shoot* elongation (mm)	Vigour index
				Pre -emergence	Post –emergence			
1	Bavistin	2.00	74.00(59.34)	3.00(9.97)	3.10(10.14)	17	33	3700
2	Indofil M-45	2.00	72.00 (58.05)	2.50 (9.10)	2.56 (9.21)	16	32	3456
3.	Vitavax	2.00	70.00 (56.79)	5.00 (12.92)	4.40 (12.11)	15	26	2870
4	Captan	2.00	82.00 (64.90)	2.50 (9.10)	2.00 (8.13)	19	38	4674
5	Thiram	2.00	76.00(6037)	4.20(11.83)	3.50(10.78)	16	33	3724
6	Control	-	65.00 (53.73)	12.00 (20.27)	10.29 (18.71)	12	27	2535
SEm+	-	1.00	0.61	0.43	-	-	65.36	
C.D. at 5%	-	(3.15)	(1.93)	(1.35)	-	-	191.81	

*Average based on 10 seedlings: Figures given in parentheses are angular transformed values

Table 2: Effect of plant extracts seed priming against *Aspergillus niger* on seed germination, pre- and post emergence mortality and vigour index (*in vitro*)

S.No.	Treatments	Dose(ml/kg seeds	Percent germination	Per cent mortality		Root* length (mm)	Shoot* length (mm)	Vigour index
				Pre -emergence	Post -emergence			
1.	Neem (*Azadirachta indica*)	10.00	74.00 (59.34)	2.00 (8.13)	3.50 (10.78)	16	36	3848
2.	Tulsi (*Ociumum sancium)*	10.00	72.50 (58.37)	5.00 (12.92)	6.80 (15.12)	14	30	3190
3.	Garlic *(Allium sativum)*	10.00	74.50 (59.67)	1.00 (5.74)	1.50 (7.03)	17	38	4097
4.	Satyanashi (*Argemone mexicana*)	10.00	70.00 (56.79)	7.50 (15.89)	9.50 (17.95)	13	26	2730
5.	Doddar (*Cuscuta sp.*)	10.00	67.00 (54.94)	11.00 (19.37)	10.30 (18.72)	14	26	2680
6.	Control	-	64.00 (53.13)	13.00 (21.13)	11.40 (19.73)	13	25	2304
SEm+	-	1.01	0.28	0.29	-	-	72.17	
C.D. at 5%	-	(3.19)	(0.88)	(0.92)	-	-	210.01	

* Average based on 10 seedlings; Figures given in parentheses are angular transformed values

Table 3.Effect of bioagents as seed treatment against *Aspergillus niger* on seed germination, pre-and post emergence mortality and vigour index (*in vitro*)

S.No.	Treatments	Dose (g/kg)	Percent germination	Per cent mortality		Root* length (mm)	Shoot* length (mm)	Vigour index
				Pre-emergence	Post- emergence			
1.	*Trichoderma harzianum*	4.00	76.00 (60.67)	4.00 (11.54)	5.80 (13.94)	19.00	39	4408.00
2.	*Trichoderma viride*	4.00	74.45 (59.64)	8.50 (16.95)	8.20 (16.64)	18.00	36	4220.30
3.	*Pseudomonas pituda*	4.00	69.50 (56.48)	11.00 (19.37)	10.70 (19.09)	17.00	34	3544.50
4.	*Microbacteria paraoxidens*	4.00	68.00 (55.55)	10.90 (19.28)	13.30 (21.39)	16.00	31	3196.00
5.	*Pseudomonas fluorescens*	4.00	71.50 (57.73)	9.50 (17.95)	10.04 (18.47)	15.00	29	3146.00
6.	Control		63.00 (52.83)	14.20 (22.14)	13.40 (21.47)	13.00	26	2457.00
SEm+	-	1.11	0.35	0.36	-	-	53.14	
C.D. at 5%	-	(3.51)	(1.10)	(1.12)	-	-	159.81	

* Average based on 10 seedlings; Figures given in parentheses are angular transformed value

(4.00/5.80%) followed by *T. viride*(8.21/8.20%) while it was minimum in FL-18 bioagent treated seeds (10.90\13.30%) in comparison to control (14.20/13.40%), respectively. The vigour index observed to significantly higher in seed treated with *Trichoderma harzianum* (4408) and *T. viride* (4220.30) in comparison to *Pseudomonas putida* (3544.50), *Microbacteria paraoxidens* (3196.00) and *Pseudomonas flourescens* (3146.00). Minimum vigour index was observed in control (2457.00) and significant difference was also observed in vigour index in seed treated with all bioagents in comparison to control. Studies on the control of seed borne fungi and to better plant stand through the application of antagonistic fungi and bacteria by seed treatment have been reported to be inconsistent but an alternative against the use of hazardous chemicals/fungicides in recent time, biocontrol agent are gaining importance in the plant disease management (Agarwal and Sinclair, 1987 and Jaiman, 2003). Seed treatment with *Trichoderma harzianum*in present studies reduce incidence of mean per cent mycoflora with the increase in seed germination. Seed treatment with *Trichoderma viride* and *T. harzianum* also reported to be reduced incidence of charcoal rot of cowpea (Ushamalini *et al.*, 1997).

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