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Management of *Alternaria* Leaf Spot and Flower Blight of Marigold (*Tagetes erecta* L.) cv. 'Crackerjack' by Applications of Fungicides and Neem Formulation

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ABSTRACT

Marigold (*Tagetes erecta* L.) cv. 'Crackerjack' is an important commercial ornamental pot and garden flower. *Alternaria zinniae*, the cause of leaf spot and flower blight, and which are seed- and air-borne in nature, are the major constraints in marigold cultivation. Seed treatment studies using 8 fungicides (mancozeb, foltaf, copper oxychloride, captan, zineb, chlorothalonil and thiram) and a neem formulation, neemicidin, were carried out against *A. zinniae*. All chemicals were effective although higher doses of some fungicides had an adverse effect on seed germination. Mancozeb and chlorothalonil effectively reduced the seed-borne infection in marigold with no adverse effect on seed germination even if applied in slightly higher doses (3.5 and 5.0 g/kg). In a field trial, mancozeb performed better than other chemicals by reducing the disease severity of leaf spot recorded in the control from 65.81 to 3.13% and with no incidence of flower blight even after 60 days. Captan and chlorothaonil were the next effective fungicides in management of the disease.

Keywords: chemical management, disease severity, doses, pathogen, seed-borne disease

INTRODUCTION

Marigold, a member of the family Asteraceae, is one of the most important flowers preferred traditionally for decoration in India. It is a native of Mexico and South America. The flower was introduced in India from Portugal during the 16th century; since then it has been naturalized in different agroclimatic zones. Although there are around 33 species of marigold, only a few are important like African and French marigold. Three species, viz. *Tagetes erecta* (African marigold), *T. patula* (French marigold) and *T. tenuifolia* (striped marigold) are the most commonly cultivated and are grown commercially all over India in around 8000-10,000 ha area with a total flower production of nearly 70,000 mt (Negi *et al*. 1998). Peruvian export of marigold meal reached 3000 t in the mid-to-late 1980s. Major importers of marigold meal and its extracts outside Latin America are North America and Western Europe, particularly Spain and Portugal (www.Prota.org). However, the world demand of *Tagetes* essential oil is about 10 t annually and *T. erecta* provides an important yellow colorant from its flower, which is produced on a significant scale in America (www.chemicalweekly.com). It is extensively used in religious and social functions and is preferred as a cut flower for internal decoration, bedding, in hanging baskets and as a loose flower for garlands. The leaves and flowers possess medicinal values having phenolic and antioxidant activities and are equally important in the pharmaceutical industry (Tripathy and Gupta 1991; Khalil *et al*. 2007). It has bioactive compounds like thiophenes that are widely employed as insecticides (particularly against *Aedes aegypti* and *Anopheles stephensi* adults and larvae; Wells *et al*. 1992), fungicides and also found to be beneficial in the control of nematode populations of *Meloidogyne* and *Pratylenchus* species when planted as an intercrop (Vasudvan *et al*. 1997). When marigold was intercropped with tomato it reduced the incidence of *Alternaria solani* (Gómez-Rodrí-

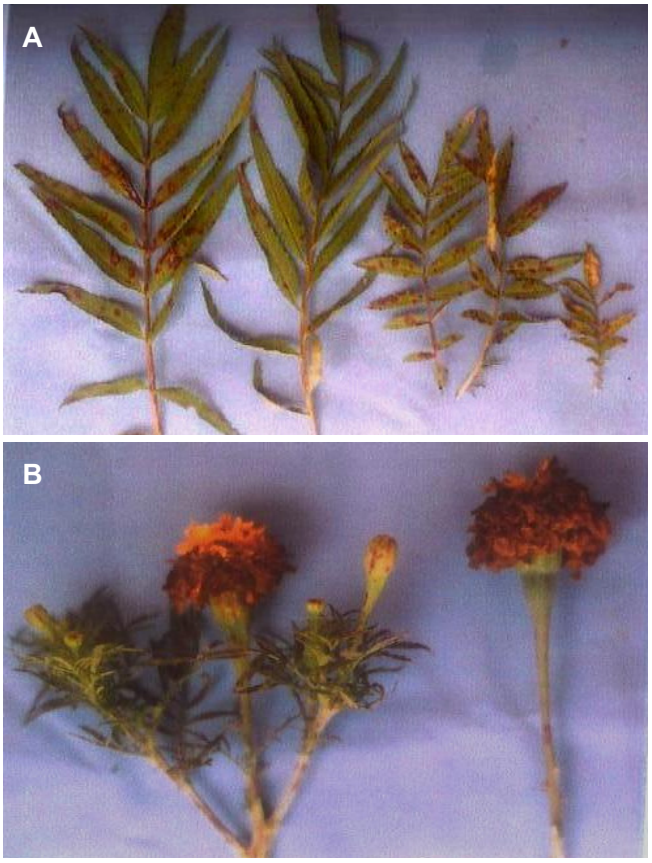

Fig. 1 Symptoms of leaf spot (A) and flower blight (B) on African marigold (*Tagetes erecta*).

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Research Note

guez *et al*. 2003). The antinemic property has been attributed to the presence of polyenes, terthienyl and bi-thienyl derivatives in roots and root exudates. It is also effective as an organic manure (Polthance and Yamazaki 1996). Marigold essential oil, which is in high demand in the perfume industry (Naik *et al*. 2003), is composed of tagetone, limonene, valeric acid and ocimene extracted from its leaves, stalks and flowers (e.g. Babu and Kaul 2007). Yellow and orange colours in marigold are due to the presence of the pigment lutein (Moehs *et al*. 2001) which is often added to poultry diets to intensify the yellow colour of egg yolks and broiler skin (Gupta 1997; Sreekala and Raghava 2003). Lutein is the main carotenoid in marigold flowers (Delgado-Vargas *et al*. 2000) which is used in the food industry (Hojnik *et al*. 2008). Carotenoids are synthesized through the non-mevalonate pathway (Dubey *et al*. 2003) that could be manipulated by genetic engineering to increase leutin accumulation or to produce new carotenoids. Transgenic *T. patula* hairy roots were used to effectively remove a colouring dye Reactive Red 198 up to 1110 mg/l (Patil *et al*. 2009).

With an increase in area, the crop has gradually become susceptible to unlimited soil-, seed- and air-borne pathogens. Leaf spot and flower blight caused by *Alternaria zinniae* have emerged as major constraints compared to other diseases by inducing 60% disease severity in African marigold (Sen 1996). The pathogen has been reported to be seed-borne in nature (Dhiman and Arora 1990). *Alternaria tegetica* causes early blight of *T. erecta* and produces phytotoxic metabolites (alternaric acid) which have an adverse effect on cell viability, fresh mass and number of cells, induced reactive oxygen species accumulation, lipid peroxidation and DNA damage on *T. erecta* cell suspension culture; this is related to the pathogenic mechanism and to phytotoxins (Qui *et al*. 2009). Foliar necrotic lesions were found on African marigold (*T. erecta*) and French marigold (*T. patula*) grown in Miyagi Prefecture, Japan (Tomioka *et al*. 2000). There are many methods of disease control but in general chemical control is considered to be the most rapid and cheapest. For example, inoculation of the *T. erecta* rhizosphere with mycorrhizal fungus *Glomus mosseae* afforded it protection from the plant pathogen *Pythium ultimum*, while the further addition of a pathogen-antagonist *Trichoderma aureoviride* allowed marigold to increase plant mass production (Calvet *et al*. 1993).

In this study, a few fungicides, including neem formulation, were evaluated as seed and foliar treatments against the disease.

MATERIALS AND METHODS

Effect of different concentration of fungicides and neem formulation on disease severity of leaf spot and seed germination of marigold

Since the pathogen is seed-borne in nature, it is essential to elucidate the pathogen associated with the seed. The causal agent was isolated and multiplied on potato dextrose agar (PDA) medium. Infected leaves and flowers of marigold (*T. erecta*) showing typical symptoms of leaf spot and flower blight (**Fig. 1**) were washed under running clean water, soaked in folds of blotting paper and cut into small pieces (2 mm) from the junction of the diseased and healthy portion with the help of a sterilized blade. These pieces were surface sterilized by dipping in 0.1% $HgCl_2$ solution for 20-30 sec and then repeatedly washed with sterilized distilled water to remove traces of $HgCl_2$ and placed on sterilized blotter filter paper to remove excess moisture. The pieces were finally transferred to PDA slants under aseptic conditions and incubated at 25°C in a BOD (biological oxygen demand) incubator (Remi CI-6S). The PDA slants were examined for fungal growth and further purified by the hyphal tip method (Soderstrøm and Erland 1996) and maintained on PDA slants at 5°C and subcultured at 30-days intervals. The isolated culture was identified on the basis of morphological characters documented by Pape (1942) and Dimock and Osborn (1942). The seeds of African marigold were inoculated with a spore suspension of approximately 150 conidia/ml of the causal agent 2 days prior to fungicide treatment. Seeds were treated with seven fungicides and one neem formulation viz. mancozeb, foltaf, copper oxychloride, captan, zineb, chlorothalonil and thiram (Indofil Chemical Co., Punjab, India) at three different doses (2.5, 3.5 and 5 g/kg) of seed while neemicidin (T. Stanes and Co. Ltd, Tamil Nadu, India) was applied 2.5, 3.5 and 5 ml/kg. The fungicide-treated seeds were sown after 1 hr in plastic pots (9 cm diameter) filled with 2 kg of formalin (6%)-sterilized soil kept for 20 days to completely release formalin fumes. 20 seeds were sown in each pot and each treatment was replicated 5 times. Untreated seeds served as the control in the same sterilized soil. After 25 days of seedling emergence, seedlings were sprayed with a spore suspension of the fungus and then kept under an artificially created humidity for 48 h to provide favourable conditions of 24 ± 1°C, > 85% relative humidity under sunlight for the development of the disease by covering the seedlings with perforated polythene bags and misting the plants regularly with an atomizer (JB128, JB Intl., Wanchai, Hong Kong). Data on the number of seedlings infected with the disease after fungicidal seed treatment were recorded and the per cent severity of leaf spot was calculated and analyzed statistically.

Protective sprays

The seedlings of African marigold were raised in nursery beds at a plant spacing of 45 cm × 45 cm with 9 plants/bed; after 45 days, seedlings were transplanted in an experimental area at the Research Farm of the Department of Mycology and Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Solan (Himachal Pradesh). The experiment was laid out in a randomized block design (RBD) with a total of 27 plots, each plot measuring 1 m^2. Nine seedlings were planted in each plot. All treatments were performed in triplicate. The recommended agronomical practices were followed and the crop was watered regularly, as per the recommendations of Bose *et al*. (2003). The data on percent disease severity of leaf spot and flower blight incidence were recorded at 20-day intervals up to 5 consecutive months immediately after the first appearance of the symptoms. Seven fungicides, including one neem formulation, at their standard recommended doses were considered for spray treatment 10 days prior to the disease by the 2nd week of June in the 2 years under study. In total, 3 sprays were applied in 1-month intervals. A 0-5 average disease severity index adopted by Hotchkiss and Baxter (1983) was used to record disease severity on leaves by examining 50 randomly selected leaves from each treatment. It was based on percentage leaf area affected where 0 = no visible symptoms, 1 = 0-10%, 2 = 11-20%, 3 = 21-30%, 4 = 31-40% and 5 = 41-50% leaf area affected. The per cent flower infection was also recorded after spraying the crop.

Statistical analyses

The experiments were designed in completely randomized and randomized block designs under pot and field conditions, respectively. The data was arc sine transformed before statistical analysis by Fisher's test ($P < 0.05$) using SPSS v. 16.0.

RESULTS AND DISCUSSION

The effect of different concentrations of fungicides and a neem formulation on disease severity and percent seed germination of African marigold were studied. Fungicides and neem formulation evaluated as seed treatment at 2.5 and 3.5 g/kg provided effective control from seed-borne infection without hampering seed germination but a few fungicides at higher doses fatally reduced seed germination and had a phytotoxic effect on seedlings, although a significantly lower incidence of disease was registered (**Table 1**). Mancozeb and chlorothalonil effectively suppressed disease at all doses: 15.4, 15.6 and 18.5% disease severity of leaf spot for mancozeb, chlorothalonil and captan, respectively, all statistically equal. Neemicidin was the least effective. Neem products such as godrej, achook, neemark, nimbicidine and neemactine acted as fungicides against many soil-borne diseases (Parmar and Ketkar 1996; Tomar and Chandel 2006;

Table 1 Effect of seed treatment with chemicals on severity of *Alternaria* leaf spot of marigold seedlings under glasshouse conditions.

Fungicide	Per cent severity of *Alternaria* leaf spot at different concentrations (g/kg seed)			Mean
	2.5 g	3.5 g	5.0 g	
Blitox-50 (copper oxychloride)	31.0 (33.8)	35.8 (36.6)	32.1 (34.5)	33.09 (35.0) e
Captan (captan)	20.0(26.6)	18.7 (25.6)	18.0 (24.3)	18.5 (25.5) b
Dithane M-45 (mancozeb)	18.8 (25.7)	17.4 (24.4)	10.2 (18.7)	15.4 (22.9) a
Foltaf (captanol)	30.0 (32.2)	28.0 (31.9)	28.4 (32.2)	28.8 (32.4) d
Kavach (chlorothalonil)	19.6 (26.3)	14.8 (22.2)	12.4 (20.6)	15.6 (23.0) a
Thiram (thiram)	37.0 (37.5)	29.1 (32.6)	25.9 (30.4)	30.7 (33.5) d
Dithane Z-78 (Zineb)	25.3 (30.2)	25.0 (30.0)	23.6 (28.3)	24.6 (29.5) c
Neemicidin (neemicidin)	46.2 (42.9)	40.4 (39.0)	35.0 (36.2)	40.5 (39.3) f
Control (untreated)	82.0 (65.0)	80.3 (63.7)	77.8 (62.0)	80.1 (63.5) g
Mean	34.5 (36.0)	32.2 (34.6)	29.2 (32.7)	

$CD_{0.05}$: Fungicide 2.10; Doses: 2.9; Fungicide x Doses: 3.02; Figures in parenthesis are arc sine transformed values; Means followed by the same letter do not differ significantly at $P < 0.05$.

Table 2 Effect of seed treatment with chemicals on germination of marigold seedlings at different concentrations.

Fungicide	Per cent germination of marigold seedlings (g/kg seed)			Mean
	2.5 g	3.5 g	5.0 g	
Blitox 50	55.7 (48.4)	63.0 (52.6)	60.0 (50.9)	59.6 (50.6) c
Captan	73.3 (58.9)	79.7 (63.3)	72.3 (58.4)	75.1 (60.2) h
Dithane M-45	82.0 (65.1)	77.7 (61.9)	72.7 (58.5)	77.1 (61.3) i
Foltaf	63.7 (53.0)	58.0 (49.6)	53.3 (47.0)	58.3 (75.1) b
Kavach	80.3 (63.8)	76.3 (61.0)	68.7 (55.7)	75.1 (54.7) h
Thiram	72.0 (58.1)	66.3 (54.5)	54.7 (47.7)	54.7 (47.7) a
Dithane Z-78	67.7 (55.4)	68.0 (55.7)	65.3 (54.0)	67.0 (55.0) g
Neemicidin	62.7 (55.4)	64.0 (53.2)	59.3 (50,4)	62.0 (52.0) f
Control (untreated)	63.0 (52.6)	59.0 (50.3)	60.7 (51.2)	60.9 (51.4) d
Mean	68.9 (56.4)	68.0 (55.8)	63.0 (52.7)	

$C.D_{0.05}$: Fungicide 0.98; Doses 1.07; Fungicide x Doses 1.1; Figures in parenthesis are arc sine transformed values; Means followed by the same letter do not differ significantly at $P < 0.05$.

Chandel and Tomar 2008; Narasimhan *et al*. 2008; Prajapati and Narain 2008), although they contain possible health risks (Boeke *et al*. 2004). Achook and nimbicidine alone and in combination with plant extracts (*Allium cepa* and *Ocimum sanctum*) were highly efficacious against *Fuasrium oxysporum* f.sp. *dianthi* when applied as a dip and soil drench treatment at 0.3% (Kumar 2004). Copper oxychloride also reduced disease severity. A higher dose (5.0 g/kg) of fungicide and neem formulation were less effective in combating disease severity compared to lower doses with an inhibitory effect on seed germination (**Table 2**): seed germination was reduced to 63.0% compared to 68.9 and 68.0% at 2.5 and 3.5 g/kg seed, respectively. Captafol, thiram and neemicidin lowered germination compared to the control at 5 g/kg seed; however, at 2.5 g/kg seed they enhanced germination. The present findings confirm those reported by Dharamvir *et al*. (1971) and Shrotri *et al*. (1983) who found mancozeb, thiram, captan and captafol best as seed treatments against seed-borne pathogens. Mayee (1991) also reported that fungi associated with seed can substantially reduce germination and hence several studies recommended seed treatments with fungicides such as thiram, metalaxyl, mancozeb and cabendazim (Kolte 1985; Chohan and Kaur 1985; Chandel *et al*. 2003). Maneb, mancozeb and zineb were efficacious against petal blight of chrysanthemum; Tank-mixed zineb, although more fungitoxic, especially in continuously high humidities, was also more phytotoxic than wettable powder and dust formulations, but this phytotoxicity could be avoided by spraying the crop at the opening bud stage and by increasing the interval between applications from 5 to 7 days (Smith 2008). In managing fairyring spot of carnation caused by *Cladosporium echinulatum*, the most effective treatments were with sodium bicarbonate and *Trichoderma virens* (Sandoval *et al*. 2009). Ram *et al*. (2002) studied the severity of Alternaria blight and yield losses in *Tagetes* sp. during 1999-2002 and reported that out of 7 fungicides tested bavistin gave the best control with minimum 6.8% average disease intensity followed by 9.5% vitavax. Use of the fungicide duojunging No. 1WP (54%), effective against Alternaria leaf spot of marigold, reduced the cost by 30% compared to other fungicides under field conditions (Gao *et al*. 2006).

Seed germination was highest again in the case of mancozeb, chlorothalonil and captan: 77.1, 75.1 and 68.7% germination while least (54.7%) was recorded in thiram, captafol and copper oxychloride (**Table 2**). Mancozeb recorded maximum seed germination and was significantly different from chlorothalonil and captan, while the latter two were statistically equally effective. The remaining treatments differed significantly with each other. At a low concentration, i.e. 2.5 g/kg, 82.0 and 83.2% seed germination was registered in mancozeb and chlorothalonil treatments, respectively.

All fungicides, including the neem formulation as a spray, effectively managed leaf spot and flower blight of marigold (**Table 3**).

A mean minimum disease severity of 10.50, 10.92 and 14.0% (all statistiacally equal) was recorded in the 2-year treatment for mancozeb-, chlorothalonil- and captan-treated plants (**Table 3**). Copper oxychloride, thiram and zineb were equally efficient in reducing disease severity. Chlorothalonil at 0.01% effectively reduced the growth of *A. zinniae* (Hotchkiss and Baxter 1983). Two fungicides (mancozeb and captan) reduced blight the most with the lowest disease incidence (6.50 and 8.50%, respectively; **Table 3, Fig. 2**). Neemicidin and captafol were least effective while chlorothalonil and thiram reduced flower blight to 20.50 and 24.00%, respectively and were found to be the next best fungicides (**Table 3**).

Neem oil is an effective and preventive fungicide used in the control of various diseases like downy and powdery mildews, rust, leaf spot, Botrytis blight, scab and flower, twig and tip blight, anthracnose and Alternaria blight (Kuepper 2003; http:/www.attra.ncat.org.html). Barnal *et al*. (2002) showed that Indofil M-45, a fungicide, inhibited mycelial growth and sporulation of *A. zinniae* by 100% only if applied 24 h immediately after infection followed by Roko and Kavach with 76.0 and 85.0% inhibition, respectively. Mazumdar (2000) also found Indofil M-45 to be superior to other fungicides, providing 87.46% control of marigold blight. Difenconzole, prochloraz or pyrifexox at 1 mg/l significantly inhibited the growth of *A. dianthicola* in

Table 3 Effect of protective sprays on severity of leaf spot and incidence of flower blight of marigold under field conditions

Treatment	Average disease severity (%) of leaf spot during year			Average flower blight incidence (%) during year		
	Year 1	Year 2	Mean	Year 1	Year 2	Mean
Blitox	29.50 (32.90	37.30 (37.58)	33.40 (35.40) b	18.50 (25.43)	45.50 (42.42)	32.50 (34.76) de
Captan	10.50 (18.4)	17.50 (24.73)	14.00 (10.50) a	4.50 (12.25)	12.50 (20.70)	8.50 (16.96) a
Dithane M-45	8.50 (16.96)	12.50 (20.70)	10.50 (18.91) a	2.50 (9.10)	10.50 (18.91)	6.50 (14.03) a
Foltaf	25.30 (30.20)	69.50 (56.48)	47.40 (43.51) d	21.50 (27.63)	55.50 (48.16)	38.50 (38.36) de
Kavach	7.35 (15.68)	14.50 (23.38)	10.92 (19.28) a	15.50 (23.19)	16.50 (27.02)	16.00 (27.00) b
Thiram	18.50 (25.48)	51.50 (45.86)	35.00 (36.67) b	16.50 (23.97)	31.50 (34.14)	24.00 (29.33) c
Dithane Z-78	18.50 (25.48)	49.50 (44.71)	34.00 (35.67) b	25.50 (30.33)	52.50 (46.43)	38.50 (38.36) de
Neemicidin	21.50 (27.63)	59.50 (50.53)	40.50 (39.52) c	21.50 (27.63)	50.50 (45.29)	36.00 (36.87) d
Control	35.50 (36.51)	75.50 (60.33)	59.50 (50.48) e	47.50 (43.57)	79.50 (63.08)	63.50 (52.83) f
Mean	21.51 (27.64)	43.03 (40.48)	-	34.11 (35.67)	30.27 (34.43)	

$C.D_{0.05}$ Disease severity 4.72; Disease incidence 5.04; Figures in parenthesis are arc sine transformed values; Means followed by the same letter do not differ significantly at $P < 0.05$.

infected carnation (Li and Wu 2002). Both saprol and triforine at 0.2% applied as a weekly or 2-week spray almost completely eradicated white rust on chrysanthemum plants (Orlikowskil and Wojdya 1987). They also reported oxycarboxin and phenapronil to be better than triforine, but the appearance of phytoxicity symptoms such as brown or dark-brown, round, oval or irregular spots on leaf margins was observed on plants treated with these fungicides.

Similarly, other approaches related to disease control are through tissue culture and resistance gene transfer. Eco-friendly approaches for virus-free plant production of African marigold was conducted *in vitro* to retrieve virus-tested cv. 'Pusa Narangi Gainda' in which different doses of nitrogen fertilizer, calcium ammonium nitrate and neem-coated urea were applied; the latter effectively reduced mosaic and *Potato virus Y* (PVY) viruses (Mehra 2003). Bacterial endophytes isolated from *T. erecta* and *T. patula*, particularly *Microbacterium esteraromaticum* and *Kocuria varian*, effectively inhibited root-lesion nematodes (*Pratylenchus penetrans*) in soils around the root zone of potatoes without affecting yield (Sturz and Kimpinski 2004).

PVY could be eliminated from plants raised from meristems 0.2-1.0 mm in size when cultured on MS medium supplemented with 4.0 mg/l kanamycin; also, when a decoction made of *Asparagus officinalis* at 5 ml/l was added to the multiplication medium PVY could not be detected (Mehra 2003). Carnation callus subjected to different concentrations of culture filtrate of *Alternaria dianthi* resulted in 11.67% cell survival at 15% selective dose of culture filtrate. Selected calli showed significantly higher levels of biochemical constituents viz., total sugars, phenols, reducing sugars and proteins as compared to non-selected calli. Banding patterns of isozymes esterase, peroxidase and polyphenol oxidase were different in selected and non selected calli. *In vitro* shoots regenerated from selected calli on MS medium supplemented with 2.0 mg/l thidiazuron (TDZ) (Mehta *et al*. 2007). Godoy-Hernández *et al*. (2009) found marigold susceptibility to *Agrobacterium tumefaciens*-mediated transformation of hypocotyls, roots, leaf sections and shoot tips aided by binary vector pCAMB1A2301 containing β-glucuronidase gene to establish a stable transformation protocol in marigold.

IMPLICATIONS AND FUTURE PERSPECTIVES

Alternaria zinniae, the cause of leaf spot and flower blight of African marigold (*Tagetes erecta*), is seed-borne in nature. There is a need to eradicate it by seed treatment with fungicides such as mancozeb, chlorothalonil, captan or zineb at optimum doses or at lower doses if it hampers seed germination. These fungicides also effectively lowered the diseases severity to a minimum damage as foliar applications in the field. Even though the disease can be controlled by chemical means, the approach is not environmentally friendly. Biotechnological means through the production of disease-free plants by tissue culture and gene transfer techniques is required for *Tagetes*.

Fig. 2 Mancozeb-treated (A) and untreated (control) plants (B) of African marigold (*T. erecta*) in a field.

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