Applied Biological Research 21(1): 28-34; (2019)
DOI: 10.5958/0974-4517.2019.00004.1

EFFECT OF SEED BIO-/CHEMO-PRIMING AND TRIAZOLE FUNGICIDE ON IMPORTANT FOLIAR DISEASES OF SUNFLOWER (*Helianthus annuus* L.)

L. Rajendran[1*], T. Selvakumar[2], C. Gopalakrishnan[3] and N. Manivannan[4]

[1,3]Department of Plant Pathology, [2]Department of Agronomy, [4]Department of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore – 641 003, Tamil Nadu (India)
*e mail: *rucklingraja@rediffmail.com*

(Received 19 May, 2018; accepted 28 November, 2018)

ABSTRACT

Sunflower (*Helianthus annuus* L.), an important edible oil seed crop, is prone to severe attack by necrosis, Alternaria leaf spot and powdery mildew diseases which lead to poor seed germination and loss of seedling vigour and yield. The present study was conducted for 3 consecutive years with the aim to manage these foliar diseases under field conditions through seed priming (bio or chemo) followed by fungicide/insecticide spray. The result revealed that among the treatments tested, seed priming with carbendazim @ 2 g + thiamethoxam @ 4 g kg^{-1} seeds followed by foliar spray of propiconazole @ 0.1% + thiamethoxam @ 0.04% twice (30 and 45 days after sowing) gave effective control of necrosis (2.27%), leaf spot (19.6 PDI) and powdery mildew (17.94 PDI) diseases with seed yield increase (1443 kg ha^{-1}) over control. This was followed by *Trichoderma viride* priming which recorded 7.1% necrosis, 38 PDI of leaf spot and 36.31 PDI of powdery mildew. Seed bio- or chemo-priming promoted higher and uniform seed germination under field conditions. Seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 4 g kg^{-1} along with two foliar sprays of propiconazole @ 0.1% + thiamethoxam @ 0.04% (30 and 45 DAS) were effective.

Key words: Seed bio-/chemo-priming, sunflower, triazole fungicide, yield

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the 3rd most important edible oil seed crop in the world and in India it is cultivated on an area of 2.4 million ha with annual production of 1.44 million t seed and productivity of 608 kg seed ha^{-1} (Ashok Kumar *et al.*, 2015). However, the crop is subject to a number of fungal, bacterial and viral diseases (Sardaru *et al*., 2013; Seiler and Gulya, 2016). Among these, the most important ones are necrosis, leaf spot and powdery mildew which cause drastic yield losses with average yield loss of 25-40% (Shankergoud *et al.*, 2006). Alternaria leaf spot [*Alternariaster helianthi* (Hansf.) E.G. Simmons (syn. *Alternaria helianthi* (Hansf.) Tubaki & Nishihara)] and sunflower necrosis (incited by tobacco streak virus belonging to Ilar virus group) diseases attack the crop during vegetative stage while powdery mildew [*Golovinomyces cichoracearum* (DC) V.P. Heluta] infects the crop from flowering to seed formation stages. Powdery mildew is an economically important disease in tropical region and was reported in high intensity (80%) during 2006-07 on *rabi* crop in India (Chander Rao *et al*., 2015). As there is no resistant variety/hybrid available against these diseases, it has become inevitable to use fungicides for disease management.

The integrated disease management through seed priming with chemical or bioagents and foliar spray of triazole fungicide has been tested under field conditions to reduce the excessive and repeated fungicidal use. Seed priming often results in rapid and uniform seedling emergence and

may be useful under adverse soil conditions (Mathre *et al*., 1994). Similarly, pelleting of sesame seeds with *Paenibacillus polymyxa* E681 significantly reduced pre- and post-emergence damping-off and also promoted plant growth and grain yield (Min Ryu *et al*., 2006). *Trichoderma* sp., besides controlling the pathogen, reportedly improve the overall crop health in terms of root length, shoot length and dry weight (Ganesan *et al*., 2007). Bijender Kumar and Kumar (2011) reported that seed treatment with *Pseudomonas fluorescens* Pf 2 (@ 0.6%) + its two foliar sprays (@ 0.6%) followed by two sprays of hinosan @ 0.1% most effectively reduced blast disease in finger millet. Seed treatment with tebuconazole 2 DS @ 1.5 g kg^{-1} seed, mancozeb 75% WP @ 3 g kg^{-1} seed, carbendazim 12% + mancozeb 63% WP @ 3 g kg^{-1} seed, were very effective in the management of soil borne diseases when used separately, with apparent yield advantage over untreated control (Jadon *et al*., 2015). However, the information on fungicide use along with bioagents is lacking. Hence, the present study was aimed to evaluate the effectiveness of bio-/chemo-priming of seeds along with two foliar sprays of Azadirachtin/*Pseudomonas*/biocides under field conditions against above diseases of sunflower.

MATERIALS AND METHODS

Field and soil conditions

The experiments were conducted in the Experimental Field of Department of Oilseeds, TNAU, Coimbatore, Tamil Nadu (India) during *kharif* 2013, 2014 and 2015. The experiment was laid in a randomized block design with three replications. The plot size was 4.2 m x 3.0 m and plant spacing 60 cm x 30 cm. The soil of the site was red loam with pH 6.7. The experimental site was low in nitrogen and phosphorus and medium in potassium. Sowing was done at 5-7 cm depth for better seed germination. Nutrients were applied as per the recommendation for the area (TNAU, 2012).

Talc-based formulation

The bioagents namely *Pseudomonas fluorescens* TNAU-Pf1 (ITCC No: BE0005) and *Trichoderma viride* Tv1 (ITCC No: 6914) were obtained from the Department of Plant Pathology, TNAU, Coimbatore (India). A loopful of *P. fluorescens* was inoculated into sterilized King's B (KB) broth and incubated on a rotary shaker at 150 rpm for 72 h at 28±2°C. After 72 h, 400 mL bacterial broth suspension containing 9 x 10^8 cfu mL^{-1}, 1 kg carrier material (talc powder), 15 g calcium carbonate (to adjust the pH to neutral) and 5 g carboxymethyl cellulose (CMC, adhesive) were thoroughly mixed under sterile conditions (Nandakumar *et al*., 2001). *T. viride* was multiplied in molasses-yeast broth (30 mL molasses; 5 g yeast; and water to make a volume of 1000 mL). The sterile broth was inoculated with an actively growing mycelial disc (9 mm) and incubated for 15 days. The biomass ($3±10^8$ cfu mL^{-1}) along with medium was incorporated into the sterile talc powder carrier material @ 50 mL suspension 100 g^{-1} talc powder and thoroughly mixed with 500 mg CMC as per Ramakrishnan *et al*. (1994). The mixture was shade-dried for 12 h and stored in polythene bags.

Bio- and chemo-priming and disease assessment

The sunflower hybrid 'CO 2' was used for the field evaluation. Sunflower seeds were surface sterilized with 1.5% NaOCl for 5 min and presoaked in sterile water for 12 h. The seeds were treated with *T. viride* @ 10 g kg^{-1} (TNAU, ITCC No. 6914) or with carbendazim 50% WP @ 2 g kg^{-1}, [Benfil, Indofil] + thiamethoxam 70 WS 4 g kg^{-1} [Slayer, GSP Crop Science Pvt. Ltd], each separately treated and incubated in moist blotter for 48 h (25-32°C) and used for sowing. Foliar spray was given 30 and 45 days after sowing as per the treatment. Seed germination, disease severity (percent disease index, PDI) for leaf spot (Mayee and Datar, 1986) and powdery mildew (Prathap Reddy *et al*., 2013) and percent disease incidence for necrosis disease were recorded by using the appropriate formula (Chander Rao, 2010). A total of 30 individual leaves were observed per treatment per replication for leaf spot and powdery mildew diseases 60 days after sowing by

random leaf sampling (15 leaves for each disease) whereas per plot observations were made for necrosis disease 30 days after sowing. For foliar application, chemical namely propiconazole 25 EC (Tilt) and thiamethoxam 25% WG (Evident 25% WG) were obtained from private agency.

The treatment combinations comprised of T_1: *Trichoderma viride* (Tv1) biopriming @ 10 g kg^{-1} seeds followed by foliar spray (FS) of propiconazole 25 EC @ 0.1% + thiamethoxam 25% WG @ 0.04% 30 and 45 days after sowing (DAS); T_2: Carbendazim 50% WP @ 2 g kg^{-1} + thiamethoxam 70 WS @ 4 g kg^{-1} seeds priming followed by FS of propiconazole 25 EC @ 0.1% + thiamethoxam 25% WG @ 0.04% twice @ 30 and 45 DAS; T_3: Tv1 biopriming @ 10 g kg^{-1} seeds followed by FS of propiconazole 25 EC @ 0.1% + Azadirachtin @ 0.15% twice @ 30 and 45 DAS; T_4: Carbendazim 50% WP @ 2g kg^{-1} + thiamethoxam 70 WS @ 4 g kg^{-1} seeds priming followed by FS of propiconazole 25 EC @ 0.1% + Azadirachtin @ 0.15% twice @ 30 and 45 DAS; T_5: Tv1 @ 10 g kg^{-1} biopriming followed by foliar spray of *P. fluorescens* @ 0.5% + Azadirachtin @ 0.15% 30 and 45 DAS; T_6: Tv1 @ 10 g kg^{-1} + thiamethoxam 70 WS @ 4 g kg^{-1} seed treatment followed by FS of propiconazole 25 EC @ 0.1% + thiamethoxam 25% WG @ 0.04% 30 and 45 DAS; and T_7: Untreated control.

Percent disease incidence (PI) for necrosis was worked out as under:

$$\text{Percent disease incidence (PI)} = \frac{\text{No of infected plants}}{\text{Total number of plants observed}} \times 100$$

The per cent disease index (PDI) for leaf spot and powdery mildew was calculated as per the formula given by Wheeler (1969).

$$\text{PDI} = \frac{\text{Sum of individual disease ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

For Alternaria leaf spot disease incidence 0-9 scale of Mayee and Datar (1986) was adopted wherein 0 = no symptoms on leaf, 1 = small, circular, scattered brown spots covering $\leq$1% leaf area, 3 = dark brown spots enlarging & covering 1-10% leaf area, 5 = dark brown spots enlarging, target like appearance covering 11-25% leaf area, 7 = dark brown spots, coalescing with target like appearance covering 26-50% leaf area, and 9 = dark brown spots, uniform, coalescing covering $\geq$51% leaf area. For powdery mildew 0-9 scale of Pratap Reddy *et al*. (2013) was followed wherein, 0 = no symptoms on leaf, 1 = powdery mildew specks covering $\leq$1% leaf area, 3 = powdery lesions covering 1-10% leaf area, 5 = enlarged powdery lesions covering 11-25% leaf area, 7 = powdery lesions coalesced to form big patches covering 26-50% leaf area; and 9 = powdery patches covering $\geq$51% leaf area and severe defoliation

Seed yield and benefit cost ratio

At harvest, sunflower heads were weighed as per replications separately and average weight calculated. After threshing and winnowing, seed weight of each replication was recorded and yield ha^{-1} computed by using net plot yield data. Benefit cost ratio is an important criterion to show the return to investment. It was worked out by dividing gross average income ha^{-1} with average cost ha^{-1}.

Statistical analysis

The data was statistically analyzed using IRRISTAT version 92 developed by IRRI Biometrics unit, Philippines. The percentage values of disease index were arcsine transformed. The data was subject to ANOVA at 5% significance level and means compared by DMRT (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The priming of sunflower seeds either with *Trichoderma* or with carbendazim + thiomethoxam significantly increased seed germination as compared to the conventional seed treatment. Priming also improved uniformity in seedling emergence. During *kharif* 2013, seed biopriming with *T. viride* @ 10 g kg^{-1} + foliar spray of propiconazole @ 0.1% + thiamethoxam @ 0.04% twice showed

Table 1: Integrated management of important foliar diseases of sunflower (*kharif* 2013)

Treatments*	Seed germination (%)	Necrosis (%)	Leaf spot (PDI)	Powdery mildew (PDI)	Seed yield (kg ha^{-1})	B:C ratio
T_1	94.0 (76.1)	2.4 (8.8)	10.9 (23.2)	13.9 (21.9)	1379	2.7
T_2	96.0 (78.7)	2.5 (9.1)	11.4 (23.8)	12.8 (20.9)	1368	2.5
T_3	92.3 (74.0)	2.7 (9.4)	12.6 (24.9)	14.1 (22.0)	1348	2.5
T_4	91.6 (73.3)	2.7 (9.4)	11.3 (23.6)	12.3 (20.5)	1337	2.4
T_5	91.0 (72.6)	3.3 (10.5)	17.8 (27.1)	18.1 (25.1)	1229	2.5
T_6	85.0 (67.3)	2.6 (9.3)	12.9 (25.3)	14.5 (22.3)	1257	2.1
T_7	72.3 (58.3)	7.3 (15.7)	23.4 (29.4)	27.4 (31.6)	1102	1.7
S.E.	1.9	0.36	0.48	0.55	23.99	
$CD_{0.05}$	4.1	0.79	1.1	1.2	52.28	

*T_1: Biopriming of *Trichoderma viride* (Tv1) @ 10 g kg^{-1} seed followed by (fb) foliar spray (FS) of propiconazole 25 EC @ 0.1% + thiamethoxam 25% WG @ 0.04% [twice, 30 and 45 days after sowing (DAS)]; T_2: Chemopriming of carbendazim 50% WP @ 2 g kg^{-1} + thiamethoxam 70 WS @ 4 g kg^{-1} seed fb FS of propiconazole 25 EC @ 0.1% + thiamethoxam 25% WG @ 0.04% (twice, 30 and 45 DAS); T_3: Tv1 biopriming @ 10 g kg^{-1} seed fb FS of propiconazole 25 EC @ 0.1% + Azadirachtin @ 0.15% (twice, 30 and 45 DAS); T_4: Chemopriming of carbendazim 50% W.P @ 2 g kg^{-1} + thiamethoxam 70 WS @ 4 g kg^{-1} seed fb FS of propiconazole 25 EC @ 0.1% + Azadirachtin @ 0.15% (twice, 30 & 45 DAS); T_5: Tv1 biopriming @ 10 g kg^{-1} fb FS of *Pseudomonas fluorescens* @ 0.5% + Azadirachtin @ 0.15% (twice, @ 30 & 45 DAS); T_6: Tv1 biopriming @ 10 g kg^{-1} + chemopriming of thiamethoxam 70 WS @ 4 g kg^{-1} seed treatment fb FS of propiconazole 25 EC @ 0.1% + thiamethoxam 25% WG @ 0.04% (twice, 30 and 45 DAS); T_7: Untreated control; Values are means of three replications and the values in parenthesis are arcsine transformed value

effective control of all the three diseases with highest seed yield of 1379 kg ha^{-1} and highest B:C ratio ratio of 2.7 (Table 1). This was followed by seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 4 g kg^{-1} plus foliar spray of propiconazole @ 0.1% + thiamethoxam @ 0.04%. In *kharif* 2014, seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 0.4 g kg^{-1} plus foliar spray of propiconazole @ 0.1% + thiamethoxam @ 0.04% twice gave highest seed yield (1393 kg ha^{-1}) and highest B:C ratio of 2.5 (Table 2). In *kharif* 2015, the disease severity was lowest in treatment seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 4 g kg^{-1} plus foliar spray of propiconazole @ 0.1% + thiamethoxam @ 0.04% twice which showed 2.5% necrosis, 35.6 PDI in case of Alternaria leaf spot, 23.5 PDI in case of powdery mildew (Table 3). This treatment gave the seed yield of 1567 kg ha^{-1} with B:C ratio of 2.22. The three years pooled data analysis revealed that seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 4 g kg^{-1} gave higher seed germination (95%) as compared to 80.4% in control (Table 4). Shirshikar (2008) has reported that the sunflower seeds treated with imidacloprid (Gaucho 70 WS @ 5 g kg^{-1}) + spray with imidacloprid (Confidor 200 SL, 0.05%, 3 sprays at 15, 30 & 45 DAS) and bordered with sorghum, had minimum incidence

Table 2: Integrated management of important foliar diseases of sunflower (*kharif* 2014)

Treatments*	Seed germination (%)	Necrosis (%)	Leaf spot (PDI)	Powdery mildew (PDI)	Seed yield (kg ha^{-1})	B:C ratio
T_1	86.7 (68.61)	2.3 (8.72)	11.4 (19.73)	19.2 (25.99)	1385	2.5
T_2	92.0 (73.57)	1.7 (7.49)	11.9 (20.18)	17.6 (24.80)	1393	2.5
T_3	88.3 (70.00)	2.3 (8.72)	12.5 (20.70)	18.5 (25.47)	1326	2.3
T_4	86.7 (68.61)	2.7 (9.46)	11.1 (19.46)	15.3 (23.03)	1355	2.4
T_5	84.3 (66.66)	3.7 (11.09)	16.8 (24.20)	20.4 (26.85)	1238	2.4
T_6	88.0 (69.73)	2.7 (9.46)	13.0 (21.13)	19.3 (26.06)	1241	2.3
T_7	79.0 (62.73)	7.3 (15.68)	23.7 (29.13)	34.3 (35.85)	1137	1.9
S.E.	0.92	0.42	0.98	1.31	20.20	
$CD_{0.05}$	4.15	1.65	2.98	2.15	51.56	

*See Table 1 for treatment details;
Values are means of three replications and the values in parenthesis are arcsine transformed value

Table 3: Integrated management of important foliar diseases of sunflower (*kharif* 2015)

Treatments*	Seed germination (%)	Necrosis (%)	Leaf spot (PDI)	Powdery mildew (PDI)	Seed yield (kg ha^{-1})	B:C ratio
T_1	96.0 (78.72)	3.0 (9.88)	45.66 (42.08)	25.00 (30.01)	1549	1.66
T_2	97.0 (80.45)	2.6 (9.36)	35.56 (36.61)	23.50 (28.99)	1567	1.68
T_3	96.3 (79.13)	4.6 (12.46)	49.23 (48.07)	27.33 (31.51)	1350	1.37
T_4	95.3 (77.64)	4.0 (11.48)	55.33 (44.56)	27.16 (31.40)	1319	1.34
T_5	93.6 (75.56)	4.3 (12.00)	55.13 (47.95)	25.50 (30.33)	1545	1.62
T_6	93.0 (74.82)	3.0 (9.88)	57.50 (49.32)	29.00 (32.58)	1448	1.55
T_7	90.0 (71.58)	6.6 (14.93)	67.0 0(54.75)	47.16 (43.38)	1062	1.23
SE	1.54	1.09	1.36	0.92	202.6	
$CD_{0.05}$	3.36	2.37	2.97	2.01	441.5	

*See Table 1 for treatment details

Values are means of three replications and the values in parenthesis are arcsine transformed value

incidence of sunflower necrosis. Jeffrey (1984) in *in vitro* studies found seed treatment with chlorothalonil and thiabendazole effective in completely inhibiting the growth of *A. helianthi*. In present study, seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 4 g kg^{-1} plus foliar spray of propiconazole @ 0.1% + thiamethoxam @ 0.04% 30 & 45 days after sowing recorded less incidence of 2.27% necrosis, less severity of leaf spot (9.6) and powdery mildew (17.94) with higher seed yield (1443 kg ha^{-1}) as compared to other treatments. In control, 7.1% necrosis, 38.0 PDI of leaf spot and 36.31 PDI of powdery mildew with seed yield of 1100 kg ha^{-1} was recorded. This is in accordance with Rao and Negeshwar Rao (1997) who found that three sprays of mancozeb on sunflower at 30, 45 and 60 DAS effective in controlling Alternaria leaf spot disease with significant yield increase in 'Morden' variety. Mesta *et al.* (2003) reported that hexaconazole was an effective fungicide against Alternaria blight of sunflower. For the management of powdery mildew, the application of wettable sulphur dust at 25-30 kg ha^{-1} at symptom initiation time or karathane (0.2%) or benlate (0.2%) spray effectively control the disease. Under high incidence of powdery mildew, two sprays of difenoconazole (0.05%) or propiconazole (0.1%) at 45 and 60 DAS effectively reduced disease severity by 46-87% and increased seed yields up to 31% over control (Chander Rao *et al*., 2015). The fungicide mancozeb + carbendazim (SAAF) evaluated against *A. helianthi* under field conditions effectively controlled Alternaria blight of sunflower (Waghe *et al.*,

Table 4: Integrated management of important foliar diseases of sunflower under field conditions (3 years pooled data)

Treatments*	Seed germination (%)		Necrosis (%)		Leaf spot (PDI)		Powdery mildew (PDI)		Seed yield (kg ha^{-1})	
T_1	92.2		2.56		22.6		19.38		1438	
T_2	95.0		2.27		19.6		17.94		1443	
T_3	92.3		3.22		24.7		19.96		1341	
T_4	91.2		3.13		25.9		18.23		1337	
T_5	89.6		3.77		29.9		22.42		1337	
T_6	88.6		2.76		27.8		20.93		1315	
T_7	80.4		7.10		38.0		36.31		1100	
S.E.	0.92		0.42		0.98		1.31		20.20	
$CD_{0.05}$	4.15		1.65		2.98		2.15		51.56	
	SE	$CD_{0.05}$	SE	$CD_{0.05}$	SE	$CD_{0.05}$	SE	$CD_{0.05}$	SE	$CD_{0.05}$
Treatment (T)	0.72	2.07	0.26	0.75	0.65	1.87	0.43	1.22	48.4	137.6
Seasons (S)	0.47	1.35	0.17	0.49	0.43	1.22	0.28	0.80	31.6	90.1
T x S	1.26	3.58	0.45	1.30	1.14	3.24	0.74	2.12	83.8	238.4
CV (%)	2.42		22.34		7.33		5.84		10.91	

*See Table 1 for treatment details

2015). The efficacy of fungicides, botanicals and bioagents in controlling Alternaria blight has earlier been reported (Rao, 2006; Mathivanan and Prabavathy, 2007; Arunakumara *et al*., 2010). The present study revealed that seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 4 g kg^{-1} promotes higher germination along with foliar spray of propiconazole @ 0.1% + thiamethoxam @ 0.04% twice 30 and 45 DAS was effective against necrosis, leaf spot and powdery mildew of sunflower.

Conclusion: Seed priming with carbendazim @ 2 g kg^{-1} + thiamethoxam @ 4 g kg^{-1} promoted higher seed germination. The seed priming plus two foliar sprays of propiconazole @ 0.1% + thiamethoxam @ 0.04% (30 and 45 DAS) effectively managed necrosis, leaf spot and powdery mildew in sunflower. The higher seed yield in this treatment was at par with *Trichoderma viride* (Tv1) biopriming @ 10 g kg^{-1} seeds plus foliar spray of propiconazole 25 EC @ 0.1% + thiamethoxam 25% WG @ 0.04% twice @ 30 and 45 DAS.

Acknowledgements: The authors gratefully acknowledge the financial support provided by AICRP-Sunflower and Biocontrol, FIST Laboratory at the Department of Plant Pathology, TNAU, Coimbatore (India).

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