

BIO-PRIMING OF SEEDS: A POTENTIAL TOOL IN THE INTEGRATED MANAGEMENT OF ALTERNARIA BLIGHT OF SUNFLOWER

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SUMMARY

Of the different integrated seed treatment options tested for the management of Alternaria blight of sunflower, highest benefit was obtained in the seed treatment with Carbendazim+Iprodione (Quintal) at 0.3% in water along with hexaconazole foliar spray (0.1%) followed by seed treatment with *Pseudomonas fluorescens* (0.8%) in jelly+hexaconazole foliar spray. Efficacy of *Pseudomonas fluorescens* as seed dresser can be enhanced by bio-priming the sunflower seeds for the effective and eco-friendly management of Alternaria blight of sunflower.

Key words: sunflower, Alternaria blight, integrated seed treatment options, bio-priming

INTRODUCTION

Alternaria blight is an important fungal disease of sunflower in India and estimates of yield losses due to this disease range up to 80%. The pathogen spreads through plant debris, seed and air and causes severe leaf spot resulting in premature defoliation and stem breakage. Alternaria leaf blight reduces the average flower size, number of seeds per plant, seed yield per plant, seed weight and percentage filling of seed. Average yield and oil losses in India ranged from 28 to 80% and 31 to 34%, respectively (Balasubrahmanyam and Kolte, 1980). Hiremath *et al.* (1993) observed that the infection of *Alternaria helianthi* reduced seed germination by 35% compared with apparently healthy seeds. Integration of chemicals, plant extracts, biotic agents along with priming agents for managing plant diseases has been considered as a novel approach, as it requires low amounts of chemicals, reducing the cost of control and pollution hazards while causing minimum interfer-

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ence with biological equilibrium (Papavizas, 1973). The use of fungicides, seed dressing chemicals, bioagents or botanicals with priming agents has become an inevitable method of disease control, particularly in sunflowers and in the absence of resistant cultivars. The infusion of fungicides into dormant seeds is a useful and efficient means of plant disease control. Acetone, dichloromethane (DCM) and polyethylene glycol (PEG) have been used to infuse systemic fungicides into seeds of a variety of crops. The infusion of systemic fungicides into soybean seeds with acetone has been shown to control damping off caused by *Phytophthora megasperma* var. *sojae* Hildebr. (Papavizas and Lewis, 1976). Seed treatment with biocontrol agents along with priming agents may serve as an important means of managing many soil and seed-borne diseases, the process often known as "bio-priming". The bio-priming seed treatment developed for control of *Pythium* seed rot of sh2 sweet corn combines microbial inoculation with preplant seed hydration. Bio-priming involves coating seed with a bacterial biocontrol agent such as *Pseudomonas aureofaciens* Kluyver AB254 and hydrating for 20 h under warm conditions (23°C) in moist vermiculite or on moist germination blotters in a self-sealing plastic bag. The seeds are removed before radicle emergence (Callan *et al.*, 1990).

Bio-priming process had potential advantages over simple seed coating with *P. aureofaciens* Kluyver AB254. Seed priming often results in more rapid and uniform seedling emergence and may be useful under adverse soil conditions. Sweet corn seedling emergence in *Pythium*-infested soil was increased by AB254 at a range of soil temperatures, but emergence at 10°C was slightly higher from bio-primed seeds than from seeds coated with the bacterium (Mathre *et al.*, 1994). Accordingly, the following study was undertaken to see the efficacy of integrated seed treatment options for the management of Alternaria blight of sunflower.

MATERIALS AND METHODS

A field experiment was conducted during October 2004, at the Main Agricultural Research Station, College of Agriculture, Dharwad, Karnataka, India, to see the efficacy of integrated seed treatment options involving a seed dressing fungicide, bioagent and botanical in different priming agents along with foliar spray of 0.1% Hexaconazole at 40 DAS (days after sowing), for the management of Alternaria blight in sunflower. A sunflower hybrid KBSH-44 was used for the study under irrigated conditions. All other recommended practices were followed and applied in all treatments.

Seeds of the sunflower hybrid KBSH-44 were treated with carbendazim+ iprodione (Quintal) at 0.3% along with different organic solvents as priming agents such as polyethylene glycol (PEG 6000), acetone, dichloromethane (DCM) and glycerol and compared with a treatment with water. *Pseudomonas fluorescens* Migula was used for bio-priming of seeds with priming agents such as vermiculite, jelly, moist blotters, salicylic acid and compared with direct seed treatment. One hundred

grams of sunflower seeds (the cultivar KBSH-44) were soaked for 24 h at $25\pm 2^{\circ}\text{C}$ in 500 ml solutions of acetone, glycerol, dichloromethane, polyethylene glycol (30% v/v) and water containing 0.3% of Quintal separately and dried under shade.

In bio-priming, hundred grams of sunflower seeds (the cultivar KBSH-44) were treated with the bacterial biocontrol agent *P. fluorescens* using priming agents such as vermiculite, jelly and moist blotters. After presoaking of seeds in sterile distilled water, seeds were coated with powder formulation of *P. fluorescens* at 0.8% concentration along with moist vermiculite in the proportion of 3:1 (3 parts of vermiculite to 1 part of seed) and mixed thoroughly to provide uniform coating. The seeds were dried in shade and stored at $25\pm 2^{\circ}\text{C}$ for 24 h in a self-sealing plastic bag before being tested under field conditions. For bio-priming in "Jelly" (a commercial preparation containing water absorbent, an organic polymer that absorbs, stores and then keeps releasing the moisture slowly), 10 g of jelly was poured into 500 ml of cold sterilized water and kept in water for 5 to 6 hours till the small pieces turned into sparkling jelly cubes. After pre-soaking of seeds in sterilized water, seeds were coated with powder formulation of *P. fluorescens* at 0.8% concentration along with jelly cubes in 3:1 proportion as stated earlier. Such treated seeds along with the jelly cubes were stored in open beakers or in self-sealing plastic bags at $25\pm 2^{\circ}\text{C}$ for 24 h before being tested in field conditions. For salicylic acid treatment, seeds were imbibed in concentration of 15 mM salicylic acid for 15-20 minutes and washed in sterile water to remove the traces of acid, then air-dried and stored as stated earlier. Seeds were also treated with neem-leaf extract in combination with chemicals and bioagents at 10% concentration. The experiment was laid out in a randomized block design with three replications. The treatments were randomly allotted to the plots. A plot size of 3.0×3.0 m was maintained with spacing of 60×30 cm. Observations on blight disease incidence at 45, 60 and 75 days after sowing were recorded by scoring five plants in each treatment on a 0 to 9 scale of Mayee and Datar (1986) and percent disease index (PDI) was calculated using a formula given by Wheeler (1969)

$$\text{PDI} = \frac{\text{Sum of numerical disease ratings}}{\text{No. of plants/leaves observed}} \times \frac{100}{\text{Maximum disease rating value}}$$

Head diameter, test weight (100-seed weight) and yield (quintal/ha) were also recorded. For benefit-cost ratio (BCR), total cost incurred for application of each treatment (including cost of chemicals and labor) was calculated. Additional benefit due to increased yield in each treatment over control was also worked out and benefit-cost ratio was calculated by dividing total cost with additional benefit.

RESULTS AND DISCUSSION

Results of this study (Table 1 and Figures 1 and 2) indicated that though the seed treatment with carbendazim+iprodione at 0.3% in poly ethylene glycol along

Table 1: Field evaluation of integrated seed treatment options with priming agents for the management of *Alternaria* leaf blight of sunflower

Tr. No	Treatment	Percent disease index at			Head diameter (cm)	Test weight (G)	Yield (q/ha)	B: C ratio
		45 DAS	60 DAS	75 DAS				
T ₁	QAH*	42.18 (40.51)*	55.00 (47.87)	67.34 (55.15)	16.00	3.41	13.97	2.17
T ₂	QDH	40.7 (39.63)	52.54 (46.45)	62.30 (52.32)	16.16	3.22	15.54	3.46
T ₃	QGH	38.23 (38.13)	56.39 (48.73)	63.39 (53.04)	17.16	3.43	15.08	2.63
T ₄	QPH	19.24 (25.53)	28.86 (32.49)	37.74 (37.89)	25.00	5.51	17.12	7.94
T ₅	QWH	22.69 (28.42)	35.27 (36.45)	42.18 (40.51)	23.00	4.30	16.1	11.66
T ₆	Q.H.	24.91 (29.88)	38.97 (38.62)	47.60 (43.62)	23.00	4.03	15.08	8.98
T ₇	PF+H	40.7 (39.63)	49.58 (44.75)	56.98 (49.02)	19.33	3.8	13.33	4.41
T ₈	PFB LH	39.22 (38.76)	46.48 (43.05)	52.54 (46.45)	19.43	3.78	15.08	3.49
T ₉	PFVH	36.26 (37.00)	43.66 (41.36)	48.59 (44.19)	20	5.01	15.27	6.24
T ₁₀	PFSAH	46.12 (42.78)	52.43 (46.45)	62.90 (52.61)	16.5	3.51	13.53	4.92
T ₁₁	PFJelly H	29.60 (32.96)	39.22 (38.76)	45.63 (42.43)	23.5	5.45	15.92	10.53
T ₁₂	SAH	48.46 (44.17)	56.48 (48.73)	63.88 (53.19)	16	3.46	12.59	2.46
T ₁₃	QSAH	43.16 (41.07)	61.42 (50.75)	68.82 (56.04)	18.5	3.89	13.18	3.97
T ₁₄	NH	44.15 (41.64)	57.47 (49.51)	65.86 (54.25)	16.33	3.62	14.99	8.76
T ₁₅	NQH	40.70 (39.62)	50.56 (45.21)	67.34 (55.14)	18.86	4.43	15.27	9.38
T ₁₆	NSAH	43.16 (41.07)	61.42 (51.72)	68.82 (56.05)	16.83	3.66	13.56	4.98
T ₁₇	NPFH	44.64 (41.91)	59.94 (50.73)	64.97 (54.33)	18.60	4.10	15.04	8.85
T ₁₈	Hexaconazole alone	44.76 (41.95)	62.00 (52.06)	64.71 (53.55)	22.83	3.45	13.36	4.52
T ₁₉	Control	61.17 (51.58)	67.83 (55.79)	76.22 (60.80)	13.33	3.18	11.66	
	S.Em ±	0.6410	0.6705	0.6187	0.3410	0.098	0.153	
	CD @ 5%	2.45	2.57	1.682	1.311	0.376	0.589	

* Figures in parentheses indicate arcsine-transformed values

T ₁	QAH	: Quintal+acetone+hexaconazole spray
T ₂	QDH	: Quintal+dichloromethane+hexaconazole spray
T ₃	QGH	: Quintal+glycerol+hexaconazole spray
T ₄	QPH	: Quintal (0.3%) + P.E.G. (30%) + hexaconazole spray (0.1%)
T ₅	QWH	: Quintal+water+hexaconazole spray
T ₆	Q.H.	: Quintal+hexaconazole spray
T ₇	PF+H	: Direct seed treatment - <i>Pseudomonas fluorescens</i> + hexaconazole spray
T ₈	PFB LH	: <i>Pseudomonas fluorescens</i> + hydrating in moist blotter + hexaconazole spray
T ₉	PFVH	: <i>Pseudomonas fluorescens</i> + vermiculite + hexaconazole spray
T ₁₀	PFSAH	: <i>Pseudomonas fluorescens</i> + salicylic acid + hexaconazole spray
T ₁₁	PFJelly H	: <i>Pseudomonas fluorescens</i> + jelly + hexaconazole spray
T ₁₂	SAH	: Salicylic acid + hexaconazole spray
T ₁₃	QSAH	: Quintal+salicylic acid+hexaconazole spray
T ₁₄	NH	: Neem leaf extract+hexaconazole spray
T ₁₅	NQH	: Neem leaf extract+Quintal+hexaconazole spray
T ₁₆	NSAH	: Neem leaf extract+salicylic acid+hexaconazole spray
T ₁₇	NPFH	: Neem leaf extract+ <i>Pseudomonas fluorescens</i> + hexaconazole spray
T ₁₈	Hexaconazole alone	: Hexaconazole alone
T ₁₉	Control	: Untreated

with foliar spray of hexaconazole recorded least percent disease indexes of 19.24, 28.86 and 37.74% at 45, 60 and 75 DAS, respectively, seed treatment with carbendazim+iprodione at 0.3% in water along with foliar spray of hexaconazole was found most effective in managing Alternaria blight with maximum benefit-cost ratio. Seed treatment of carbendazim+ iprodione at 0.3% in water along with foliar spray of hexaconazole showed a disease index of 22.69% at 45 DAS, which was found on par with direct seed treatment with carbendazim+iprodione at 0.3% concentration along with foliar spray of hexaconazole. However, it showed significant difference in percent disease index at 60 and 75 DAS with disease incidence of 35.27 and 42.18%, respectively, compared with the incidences of 38.97 and 47.60% at 60 and 75 DAS, respectively, obtained by direct seed treatment with carbendazim+ iprodione. The bioagent *Pseudomonas fluorescens* performed better in "jelly" as priming agent along with foliar spray of hexaconazole, which recorded the percent disease indexes of 29.6, 39.22 and 45.63% at 45, 60 and 75 DAS, respectively, which was found to be significantly superior over the other priming agents and also over the direct seed treatment with *P. fluorescens*. The seed treatment with *P. fluorescens* in vermiculite as priming agent also showed good response, next to "jelly", with the percent disease indexes of 36.26, 43.66 and 48.59% at 45, 60 and 75 DAS, respectively, which was found to be significantly superior to direct seed treatment with *P. fluorescens*.

Effect of integrated seed treatments on yield parameters of sunflower

Seeds treated with carbendazim+iprodione in PEG recorded the highest yield of 17.12 q/ha (Table 1 and Figure 1), with test weight and head diameter of 5.51 g and 25 cm, respectively, which differed significantly over the seed treatment with carbendazim+iprodione in water which showed an average yield, head diameter and test weight of 16.1 q/ha, 23 cm and 4.30 g, respectively. Still, the highest benefit was obtained with seed treatment with carbendazim+iprodione in water with maximum benefit-cost ratio of 11.66 followed by PF Jelly H (i.e., seed treatment with *P. fluorescens* in jelly+hexaconazole foliar spray), with a benefit-cost ratio of 10.53. The direct seed treatment with carbendazim+iprodione recorded an average yield of 15.08 q/ha, which differed significantly with carbendazim+iprodione in water (16.10 q/ha) but was found to be on par with respect to test weight (4.03 g) and head diameter (23.00 cm). The seed treatment with *P. fluorescens* in "jelly" as priming agent along with foliar spray with hexaconazole recorded an average yield of 15.92 q/ha and head diameter of 23.50 cm, which was found on par with the seed treatment with carbendazim+ iprodione in water with an average yield and head diameter of 16.10 q/ha and 23 cm, respectively, which clearly indicated that bio-priming of sunflower seeds with *P. fluorescens* in the form of jelly can be used as an alternative method to seed treatment with chemicals which is ecofriendly and which avoids possible residue problems. The observed improvements due to bio-priming of sunflower seeds may be due to priming-induced quantitative changes in

biochemical content of the seeds and improved membrane integrity (Sung and Chang, 1993). This may be also due to the proliferation of the bioagent *P.fluores-*
cens in the priming medium, *i.e.*, jelly. Callan *et al.* (1990) also stated that the bac-
terial biocontrol agent may multiply substantially on seed during bio-priming.

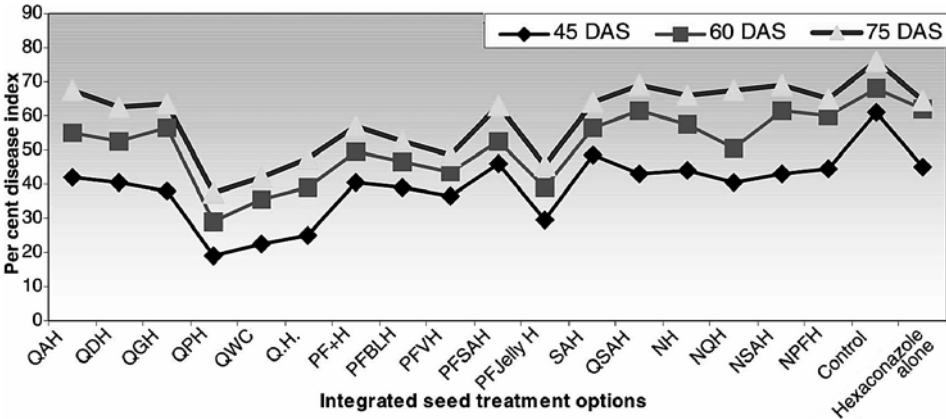


Figure 1: Effect of integrated seed treatment options with priming agents on the percent disease index (PDI) of *Alternaria* leaf blight of sunflower

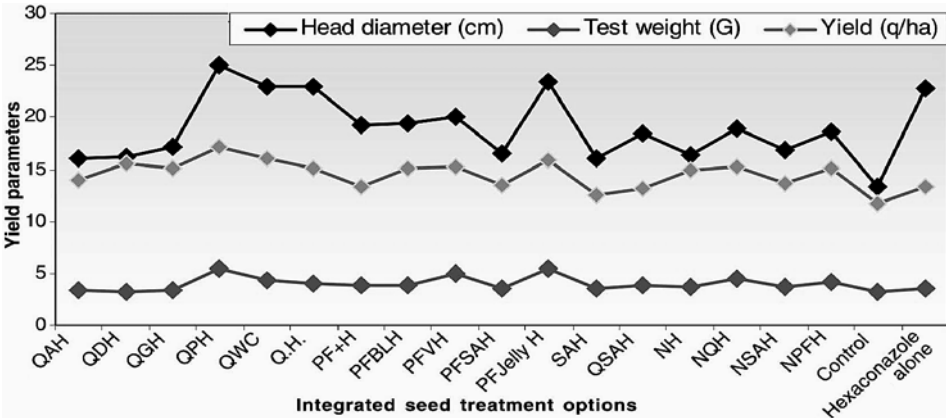


Figure 2: Effect of integrated seed treatment options with priming agents on the management of *Alternaria* leaf blight in sunflower

CONCLUSIONS

Results of the present study indicated that bio-priming of sunflower seeds with *P.fluorescens* in “jelly” can be used as an alternative method to seed treatment with chemicals which is economical, ecofriendly and which provides protection to seedlings against seed-borne infections such as *Alternaria helianthi* causing *Alternaria* leaf blight.

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BIO PELETEADO DE SEMILLAS: UNA HERRAMIENTA POTENCIAL EN EL MANEJO INTEGRADO DE LA ALTERNARIA EN GIRASOL

RESUMEN

Entre las diferentes opciones para el tratamiento integrado de semilla que se evaluaron para el manejo de la *Alternaria* del girasol, los mayores beneficios se obtuvieron con el tratamiento de Carbendazim+Iprodione (Quintal) al 0,3% en agua junto con aplicación foliar de Hexaconazole al 0,1% seguido por el tratamiento de semillas con *Pseudomonas fluorescens* (0,8%) en gel+aplicación foliar de Hexaconazole. Se puede mejorar la eficacia de *Pseudomonas fluorescens* como tratamiento de semillas a través del biopeteado para el tratamiento efectivo y ecológico de la *Alternaria* del girasol.

BIO-PRÉPARATION DE LA GRAINE: POTENTIALITÉ D'UNE PROTECTION INTÉGRALE DU TOURNESOL CONTRE LA FANURE PROVOQUÉE PAR LE CHAMPIGNON ALTERNARIA

RÉSUMÉ

Parmi les différents systèmes intégrés de traitement de semences testés pour le management de la maladie cause par *Alternaria* chez le tournesol, le bénéfice le plus important a été obtenu avec un traitement avec le mélange Carbendazim+Iprodione (Quintal) à 0.3 pour cent dans de l'eau accompagné par un spray foliaire de hexaconazole (0.1%), suivi par un traitement de semences avec *Pseudomonas fluorescens* (0.8%) dans un gel également

accompagné par le spray foliaire de hexaconazole. L'efficacité de *Pseudomonas fluorescens* pour l'enrobage des semences peut être augmentée par le bio priming des semences de tournesol pour un management efficace et respectueux de l'environnement de la maladie causée par *Alternaria* chez le tournesol.