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Bio-Intensive Disease Management Strategy as a Way to Control Pomegranate Wilt Caused by *Ceratocystis fimbriata* Ell. and Halst

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Pomegranate (*Punica granatum* L.) is one of the important fruit crops cultivated all over the world, particularly in the tropical and sub-tropics. It is affected by several diseases of which one of the most important diseases is *Ceratocystis fimbriata.* In the present study, we aimed to the management of pomegranate wilt. The management, under *in vitro* studies, captan, mancozeb, ziram, thiram, and zineb recorded maximum inhibition of mycelial growth at all concentrations (0.10%, 0.20%, and 0.30% respectively). Out of nine systemic fungicides tested, carbendazim, hexaconazole, thiophanate methyl, propiconazole, and tebuconazole showed 100 per cent inhibition at all concentrations (0.05%, 0.10% and 0.15% respectively). In case of combi-fungicide molecules, hexaconazole + zineb, carbendazim + mancozeb, trifloxystrobin + tebuconazole and

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captan + hexaconazole were found highly effective. Among bio-agents tested, *T. harzianum* (Th-R) and Diamond (*T. viride*) were found more effective as compared to other bio-control agents and inhibited maximum fungal growth (100%) of *C. fimbriata.* The fungicides and bio-agents which showed superior performance *in vitro* were selected and treatment combinations were made to develop a bio-intensive integrated management strategy against pomegranate wilt under field conditions. Field evaluation over two years indicated that three drenching of propiconazole (0.2%), Diamond (*T. viride*) (0.7 g/l) and *T. harzianum* (5g/l) at an interval of 15 days showed maximum disease control.

Keywords: Pomegranate; drenching; bioagents; bio-intensive.

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is an ancient fruit, that belongs to the family lythraceae. Pomegranate is native to Iran, where it was first cultivated in about 2000 BC and spread to the Mediterranean countries. It is cultivated in India, Iran, China, Turkey, USA, Spain, Azerbaijan, Armenia, Afghanistan, Uzbekistan, the Middle East, Pakistan, Tunisia, Israel, dry regions of Southeast Asia, Peninsular Malaysia, the East Indies, and tropical Africa. The area under pomegranate is increasing worldwide because of its hardy nature, wider adaptability, drought tolerance, higher yield levels with excellent keeping quality, and remunerative prices in domestic as well as export markets. It thrives well in dry tropics and sub-tropics and comes up very well in soils of low fertility status as well as in saline soils. India is the world's leading country in pomegranate production.

It is one of the most adaptable subtropical fruit crops. In India it is regarded as a "vital cash crop", extensively grown in Maharashtra, Karnataka, Andra Pradesh, Telangana, and Gujarat, and is picking up fast in Himachal Pradesh, Rajasthan, and Madhya Pradesh. Small areas are under cultivation in Tamil Nadu, Mizoram, Odisha, Nagaland, Lakshadweep, Jharkhand and Jammu Kashmir. total area under pomegranate in India is 1,80,640 ha out of which 1,28,650 ha is in Maharashtra only. The total production in India is 17,89,310 metric tons and 11,97,710 metric tons in Maharashtra. In Karnataka total area is 23,230 ha with production of 2,61,820 metric tonnes (http://nhb.gov.in**)**.

In Karnataka, the crop has spread across different districts *viz.,* Vijayapura, Bagalkot, Koppal, Yadgir, Raichur, Ballari, Chitradurga, Tumakuru, and Hassan. The most popular varieties suitable for processing and table use are Ganesh, Mridula, Arakta, Bhagwa (Kesar), G-137 and Khandar. Successful cultivation of pomegranate in recent years has been threatened by different pests and diseases. Bacterial blight, wilt, anthracnose, leaf spot, and root-knot nematode are important diseases. Among them, wilt caused by *Ceratocystis fimbriata* Ell. and Halst. is an emerging threat. At present the crop is severely affected by wilt pathogen and day by day the wilting severity is increasing at a faster rate. It was first noticed in some areas of Vijayapur districts of India during 1990. By 1993, the rapid spread of this disease was observed in the entire Vijayapura district. The cause was not identified until 1995; however, in 1996 the fungus *C. fimbriata* was isolated from discolored stem, root, and branch tissues on wilting plants. The disease is characterized by initial symptoms of yellowing and wilting of leaves on one to several branches leading to the death of affected plants in a few weeks. Cross sections of diseased plants revealed brown discoloration in the outer xylem from the roots to the main trunk [1].

The disease is prevalent in parts of Maharashtra, Karnataka, Telangana, Gujarat, and Tamil Nadu states [2]. Despite many factors conducive to the high severity, seedlings selection for planting, soil-borne nature, and also association with shot hole borer and plant parasitic nematodes is noticed. This might be the reason for the current rampant spread of the disease in south Indian states. Several agents are known to cause wilt in pomegranate, but *C. fimbriata* is the major cause [3], [4], hence, emphasis should be on *C. fimbriata*.

In the modern era of organic fruit production, dependence on fungicides and other chemicals is reducing. In this context, the use of antagonists as well as their combinations with fungicides to manage disease is receiving a lot of attention. Resistance-inducing rhizobacteria offer an excellent alternative in providing natural, effective, safe, persistent, and durable protection. Plants have endogenous defense mechanisms that can be induced in response to the pathogen and bio-agents. One classical biotic inducer is the plant growth-promoting bacterium, *Pseudomonas fluorescens* [5]. *Trichoderma* spp. can reduce the severity of plant diseases by inhibiting plant pathogens in the soil through its highly potent antagonistic and mycoparasitic activity. Moreover, as revealed by research in recent decades, some *Trichoderma* strains can interact directly with roots, increasing plant growth potential, resistance to disease, and tolerance to abiotic stresses [6].

Though, soil application of fungicides such as propiconazole and carbendazim has been recorded to check wilt due to *Ceratocystis fimbriata*, wilt disease epidemics are still not uncommon. There is huge concern over environmental safety due to the indiscriminate use of chemical fungicides besides their escalated costs. Management of *C. fimbriata* through soil application of fungicides is difficult because of its broad host range as well as its worldwide distribution which precludes such a strategy. Once established in the soil, it is difficult to eliminate the pathogen. Management through chemical methods leads to ill effects like residual toxicity, environmental pollution, and fungicide resistance. Although soil application with fungicides is recommended to minimize the infection at early stages, it does not give complete protection. A single method of management may not be possible to control this disease effectively. Therefore, it is necessary to develop a bio-intensive management strategy to manage the wilt disease of pomegranate.

2. MATERIALS AND METHODS

The fungicides and bio-agents which showed superior performance *in vitro* were selected and treatment combinations were made to develop bio-intensive integrated management strategy against pomegranate wilt under field conditions. The farmers orchard where (4 years old) plants showing typical initial disease symptoms at Ganjalli village of Raichur taluk planted with the most popular variety, Kesar was selected for conducting the experiment for consecutive two years during *Hastbahar* 2014 and 2015. The experiment was laid out in Randomized Block Design (RBD) with a plant spacing of 12 x 6 feet. The following treatments were formulated to find out the integrated disease management strategy using bio-agents and fungicides.

For each treatment, a plant showing typical initial wilting disease symptoms (Yellowing of leaves in 1-2 branches) was selected; fungicidal solution of treatments was prepared and drenched @ 8-10 lit. Likewise, four plants were maintained and treated to represent four replications per treatment. For preparing treatment solutions, a known quantity of fungicide and bio-agent were dissolved in water and all horticultural practices as per package of practices were followed for raising the crop by farmer.

Chart 1. List of treatments used for the study

Treatment	Particulars
T_1	Carbendazim (0.2%)* - carbendazim (0.2%)* – carbendazim (0.2%)*
T_2	Propiconazole (0.2%) – propiconazole (0.2%) – propiconazole (0.2%)
T_3	Platinum (0.7 g/l) - platinum (0.7 g/l) - platinum (0.7 g/l)
T_4	Diamond (0.7 g/l) - diamond (0.7 g/l) - diamond (0.7 g/l)
T_5	*Trichoderma harzianum* (Th-R) (5 g/l) - *T. harzianum* (Th-R) (5 g/l) - *T. harzianum*(Th-R) (5 g/l)
T_6	*Pseudomonas fluorescens* (RP-46) (5g/l) - *P. fluorescens* (RP-46) (5 g/l) - *P. fluorescens* (RP-46) (5 g/l)
T_7	Platinum (0.7 g/l) - propiconazole (0.2%) - platinum (0.7 g/l)
T_8	Diamond (0.7 g/l) - propiconazole (0.2%) - diamond (0.7 g/l)
T_9	Platinum (0.7 g/l) – carbendazim (0.2%) - platinum (0.7 g/l)
T_{10}	Diamond (0.7 g/l) – carbendazim (0.2%) - diamond (0.7 g/l)
T_{11}	Carbendazim (0.2%) - platinum (0.7 g/l) - carbendazim (0.2%)
T_{12}	Carbendazim (0.2%) - diamond (0.7 g/l) - carbendazim (0.2%)
T_{13}	Propiconazole (0.2%) - platinum (0.7 g/l) - propiconazole (0.2%)
T_{14}	Propiconazole (0.2%) - diamond (0.7 g/l) - propiconazole (0.2%)
T_{15}	Control

** First, second and third drenching were done in sequence at an interval of 15 days*

For recording wilt incidence, observations on total number of branches and number of wilted branches in each treatment were recorded. The first observation was recorded before the drenching of treatments to know the initial incidence of the disease. Later, observations on the effect of treatments were recorded from 15 days to 120 days at 15-day intervals. The fruit yield (t/ha) was also recorded and later per cent increase in yield over control was calculated.

3. RESULTS

3.1 Management Under Field Condition

Field experiments on development of bio-intensive disease management strategy against pomegranate wilt were conducted for two consecutive years during 2014 and 2015. The results on disease incidence, fruit yield, and BC ratio are presented (Table 1, Table 2, Table 3, Table 4 and Plate 1) Disease incidence (% wilting of branches)

3.1.1 I Year (2014)

The incidence of wilt was 12.6 to 13.9 per cent in different treatments before drenching of treatment combinations. The disease incidence started increasing from 15 days to till 120 days and the highest incidence (100%) was recorded in the control treatment at 105 days. Among the treatments employed, the treatment containing three drenches of propiconazole (0.2%) at 15-day intervals recorded significantly the lowest disease incidence of 10.28 per cent when compared to the rest of the treatments. However, the treatment was significantly superior to diamond (*T. viride*) (0.7 g/l) and *T. harzianum* (Th-R) (5 g/l) which were also effective in reducing the disease incidence (18.33% and 19.93%, respectively). Further, treatment combinations such as three drenching of carbendazim (0.2%), propiconazole (0.2%) - diamond (*T. viride*) (0.7 g/l) - propiconazole, propiconazole (0.2%) - platinum (*P. fluorescens*) (0.7 g/l) – propiconazole and carbendazim (0.2%) - diamond (*T. viride*) (0.7 g/l) - carbendazim (0.2%) were also effective to some extent in reducing wilt (29.98%, 32.71%, 36.55% and 39.53% respectively). The untreated control treatment showed starting incidence of 13.00 per cent at 15 days of drenching to a maximum of 100 per cent at 120 days (Table 1).

3.1.2 II Year (2015)

During *Hastbahar*, 2015**,** the wilt incidence ranged from 12.0-13.8 per cent in different treatments before the drenching of treatments. The disease incidence started increasing from 15 days to till 120 days. The highest incidence (100%) was recorded in the control treatment at 105 days. Among the treatments employed, the significantly lowest disease incidence of 10.58 per cent was recorded in the treatment of three drenchings of propiconazole (0.2%) at 15 days interval when compared to the rest of the treatments and it was significantly superior to diamond (*T. viride*) (0.7 g/l) and *T. harzianum* (Th-R) (5g/l) which were also effective in reducing the disease incidence (18.53% and 20.66%, respectively). The untreated control treatment showed a starting incidence of 13.66 per cent at 15 days of drenching to a maximum of 100 per cent at 120 days (Table 2).

3.2 Pooled Data (2014 and 2015)

In the results on pooled treated data also indicated the same trend of results as recorded in individual years. propiconazole (0.2%), three drenching at 15 days intervals showed significantly superior result in recording least disease incidence (10.53%) over all other treatments (Table 3). The next best treatments diamond (*T. viride*) (0.7 g/l) - diamond (*T. viride*) (0.7 g/l) - diamond (*T. viride*) (0.7 g/l) drenching at 15 days intervals (18.53 %), T. *harzianum* (Th-R) (5 g/l) *- T. harzianum* (Th-R) (5 g/l) - *T. harzianum* (Th-R) (5 g/l) drenching at 15 days intervals (20.66%) but significantly different from untreated control (100%).

3.2.1 Fruit yield (t/ha)

The data on the fruit yield of pomegranate was recorded during 2014 and 2015; later pooled analysis was done and given in Table 3.

3.2.2 I Year (2014)

Results on fruit yield of pomegranate during 2014 indicated that, drenching of propiconazole (0.2%) alone recorded significantly highest fruit yield (10.31 t/ha) and it was significantly different from the rest of the treatment combinations. diamond (*T. viride*) (0.7 g/l) alone was also effective in recording a fruit yield of 8.7 t/ha. Further, *T. harzianum* (Th-R) alone (5g/l) showed 8.59 t/ha (Table 3). However, no fruits were formed in untreated control due to the death of plants attributed to very high wilt incidence.

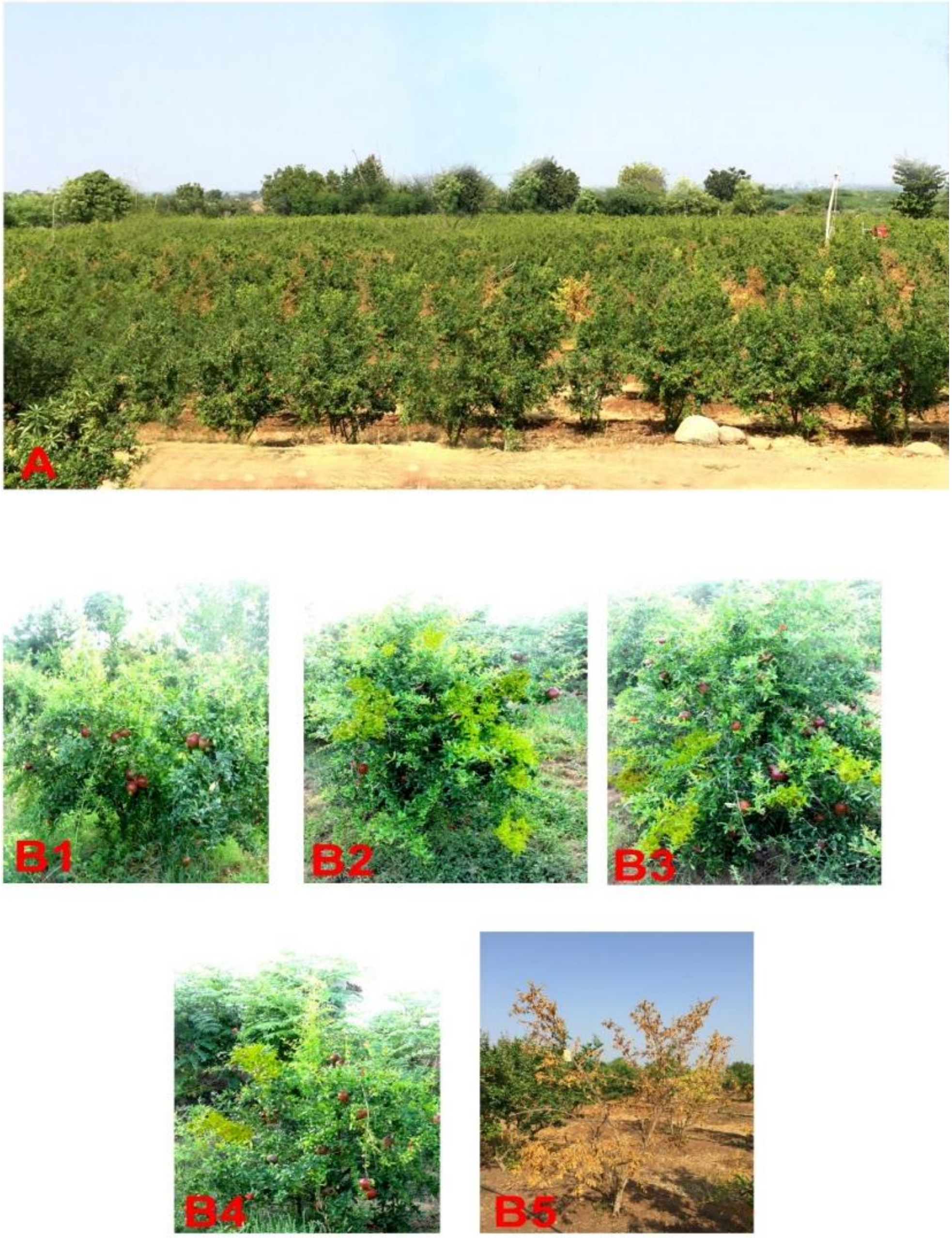

Plate 1. Management of pomegranate wilt under field conditions
A) General view of experimental plot
B) Individual treatments
B1 - T_2 (Propiconazole (0.2%) - Propiconazole (0.2%) - Propiconazole (0.2%)
B2 - T_3 (Platinum (0.7 g/l) - Platinum (0.7 g/l) - Platinum (0.7 g/l),
B3 - T_4 (Diamond (0.7 g/l) - Diamond (0.7 g/l) - Diamond (0.7 g/l))
B4 - T_5 (Trichoderma harzianum (5 g/l) – T. harzianum (5 g/l) - T. harzianum (5 g/l)
B5 - T_{15} (Control)

3.2.3 II Year (2015)

A similar trend of results was obtained in 2015 with respect to fruit yield. Data indicated that the treatment propiconazole (0.2%) alone recorded significantly highest fruit yield (10.43 t/ha) followed by diamond (*T. viride*) (0.7 g/l) (8.78 t/ha) and *T. harzianum* (Th-R) (5g/l) (8.55 t/ha) (Table 3). However, untreated control treatment recorded no fruit yield (0.00 t/ha).

Table 1. Development of bio-intensive disease management of pomegranate wilt during 2014

Treatment	Disease incidence (% wilting of branches)								
	Before drenching	After treatment (Days)							
		15	30	45	60	75	90	105	120
T_1	13.9 (21.88)	17.59 (24.79)	19.59 (26.27)	22.37 (28.23)	24.14 (29.43)	25.40 (30.26)	26.40 (30.92)	28.07 (31.99)	29.98 (33.20) *
T_2	12.7 (20.86)	18.04 (25.14)	20.09 (26.63)	20.09 (26.63)	19.05 (25.88)	17.15 (24.46)	14.13 (22.08)	12.75 (20.92)	10.28 (18.70)
T_3	13.6 (21.65)	19.16 (25.96)	24.69 (29.79)	31.52 (34.15)	37.67 (37.86)	40.90 (39.76)	42.49 (40.68)	43.39 (41.20)	44.11 (41.62)
T_4	13.5 (21.56)	19.30 (26.06)	21.20 (27.41)	24.30 (29.53)	23.98 (29.32)	23.38 (28.91)	21.80 (27.83)	19.1 (25.91)	18.33 (25.35)
T_5	13.9 (21.91)	19.69 (26.34)	22.28 (28.17)	26.06 (30.70)	26.06 (30.70)	25.15 (30.10)	23.05 (28.69)	21.3 (27.49)	19.93 (26.51)
T_6	12.6 (20.77)	18.47 (25.45)	27.81 (31.83)	35.75 (36.72)	41.62 (40.17)	45.02 (42.14)	48.44 (44.10)	53.71 (47.13)	57.08 (49.07)
T_7	13.5 (21.52)	16.88 (24.26)	21.27 (27.46)	29.81 (33.09)	33.74 (35.51)	39.95 (39.20)	44.94 (42.10)	48.37 (44.06)	51.79 (46.03)
T_8	13.0 (21.10)	17.65 (24.84)	25.68 (30.45)	31.77 (34.31)	38.97 (38.62)	42.82 (40.87)	45.34 (42.33)	47.02 (43.29)	48.27 (44.01)
T_9	13.1 (21.24)	18.68 (25.61)	25.15 (30.10)	29.84 (33.11)	36.90 (37.40)	41.33 (40.00)	48.42 (44.10)	51.02 (45.58)	52.72 (46.56)
T_{10}	13.9 (21.87)	18.73 (25.64)	23.49 (28.99)	26.96 (31.28)	32.31 (34.64)	40.04 (39.25)	47.57 (43.61)	50.44 (45.25)	53.12 (46.79)
T_{11}	12.9 (21.05)	16.63 (24.06)	21.36 (27.52)	24.08 (29.39)	30.70 (33.65)	35.26 (36.43)	38.99 (38.64)	40.71 (39.65)	42.71 (40.81)

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Treatment	Disease incidence (% wilting of branches)								
	Before drenching	After treatment (Days)							
		15	30	45	60	75	90	105	120
T_{12}	12.3 (20.49)	16.75 (24.16)	24.29 (29.53)	29.53 (32.92)	32.97 (35.04)	35.82 (36.76)	36.55 (37.20)	38.61 (38.41)	39.53 (38.96)
T_{13}	13.2 (21.31)	17.48 (24.72)	21.76 (27.81)	25.35 (30.23)	29.80 (33.08)	33.41 (35.31)	34.36 (35.88)	34.28 (35.83)	36.55 (37.20)
T_{14}	12.5 (20.67)	18.45 (25.44)	20.97 (27.25)	24.34 (29.56)	29.95 (33.18)	31.85 (34.36)	32.71 (34.89)	32.71 (34.89)	32.71 (34.89)
T_{15}	13.0 (21.09)	22.65 (28.42)	29.58 (32.94)	38.07 (38.10)	54.92 (47.82)	67.90 (55.49)	93.16 (74.84)	100.00 (90.00)	100.00 (90.00)
S.Em.±	0.69	0.86	0.80	0.98	0.68	0.76	0.83	0.67	0.82
CD at 5%	1.97	2.47	2.29	2.81	1.92	2.17	2.39	1.93	2.35

** Figures in parenthesis arc sine transformed value*

T_1 : Carbendazim (0.2%) - Carbendazim (0.2%) - Carbendazim (0.2%)
T_2 : Propiconazole (0.2%) - Propiconazole (0.2%) - Propiconazole (0.2%)
T_3 : Platinum (0.7 g/l) - Platinum (0.7 g/l) - Platinum (0.7 g/l)
T_4 : Diamond (0.7 g/l) - Diamond (0.7 g/l) - Diamond (0.7 g/l)
T_5 : T. harzianum (Th-R) (5 g/l) - T. harzianum (Th-R) (5 g/l) - T. harzianum(Th-R) (5 g/l)
T_6 : P. fluorescens (RP-46) (5 g/l)-P. fluorescens(RP-46) (5 g/l)-P. fluorescens (RP-46) (5 g/l)
T_7 : Platinum (0.7 g/l) - Propiconazole (0.2%) - Platinum (0.7 g/l)
T_8 : Diamond (0.7 g/l) - Propiconazole (0.2%) - Diamond (0.7 g/l)
T_9 : Platinum (0.7 g/l) - Carbendazim (0.2%) - Platinum (0.7 g/l)
T_{10}: Diamond (0.7 g/l) - Carbendazim (0.2%) - Diamond (0.7 g/l)
T_{11}: Carbendazim (0.2%) - Platinum (0.7 g/l) - Carbendazim (0.2%)
T_{12}: Carbendazim (0.2%) - Diamond (0.7 g/l) - Carbendazim (0.2%)
T_{13}: Propiconazole (0.2%) - Platinum (0.7 g/l) - Propiconazole (0.2%)
T_{14}: Propiconazole (0.2%) - Diamond (0.7 g/l) – Propiconazole (0.2%)
T_{15}: Control

Table 2. Development of bio-intensive diseases management of pomegranate wilt during 2015

Treatment	Disease incidence (% wilting of branches)								
	Before drenching	After treatment (Days)							
		15	30	45	60	75	90	105	120
T_1	13.80 (21.81)	18.36 (25.36)	21.71 (27.76)	24.15 (29.43)	27.60 (31.69)	29.87 (33.12)	33.32 (35.25)	33.32 (35.25)	34.51 (35.97)*
T_2	13.33 (21.41)	15.35 (23.06)	17.65 (24.84)	17.65 (24.84)	16.77 (24.17)	14.88 (22.69)	12.90 (21.05)	11.61 (19.92)	10.58 (18.98)
T_3	13.52 (21.52)	20.49 (26.86)	23.91 (29.23)	29.67 (32.98)	37.59 (37.80)	42.20 (40.49)	45.67 (42.50)	48.90 (44.37)	50.01 (45.01)
T_4	13.43 (21.50)	15.17 (22.92)	16.97 (24.33)	18.63 (25.57)	18.74 (25.65)	19.63 (26.30)	19.34 (26.09)	19.04 (25.87)	18.53 (25.50)
T_5	12.73 (20.91)	14.57 (22.44)	16.05 (23.62)	18.05 (25.14)	19.05 (25.88)	21.68 (27.75)	21.10 (27.34)	21.10 (27.34)	20.66 (27.03)
T_6	12.00 (20.27)	20.20 (26.69)	26.20 (30.78)	33.76 (35.52)	38.11 (38.12)	43.66 (41.35)	47.93 (43.81)	53.92 (47.25)	57.77 (49.48)
T_7	13.43 (21.47)	17.12 (24.42)	21.88 (27.88)	30.60 (33.58)	35.36 (36.49)	45.15 (42.22)	46.44 (42.96)	49.05 (44.46)	53.83 (47.20)
T_8	12.5 (20.68)	19.37 (26.10)	26.02 (30.64)	31.92 (34.40)	38.57 (38.39)	44.25 (41.70)	46.67 (43.09)	48.87 (44.35)	51.12 (45.64)
T_9	12.19 (20.41)	18.93 (25.76)	24.46 (29.63)	28.87 (32.50)	35.54 (36.59)	41.13 (39.89)	45.62 (42.48)	48.97 (44.41)	55.55 (48.19)
T_{10}	12.60 (20.79)	20.16 (26.68)	26.27 (30.82)	29.72 (33.03)	35.65 (36.66)	45.19 (42.24)	50.13 (45.07)	53.73 (47.14)	56.00 (48.45)
T_{11}	12.92 (20.98)	18.57 (25.52)	23.31 (28.85)	26.81 (31.17)	31.34 (34.04)	37.35 (37.67)	40.44 (39.49)	42.92 (40.93)	45.34 (42.33)

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Treatment	Disease incidence (% wilting of branches)								
	Before drenching	After treatment (Days)							
		15	30	45	60	75	90	105	120
T_{12}	12.97 (21.01)	17.55 (24.74)	24.36 (29.57)	30.19 (33.33)	33.87 (35.58)	38.41 (38.30)	39.24 (38.79)	40.53 (39.54)	41.72 (40.23)
T_{13}	13.05 (21.16)	19.19 (25.93)	23.89 (29.24)	29.95 (33.14)	34.64 (36.04)	36.94 (37.43)	38.18 (38.16)	39.25 (38.79)	40.53 (39.54)
T_{14}	13.43 (21.50)	19.29 (26.05)	22.93 (28.61)	27.65 (31.72)	32.53 (34.77)	33.60 (35.42)	36.16 (36.96)	37.23 (37.60)	38.57 (38.39)
T_{15}	13.66 (21.68)	21.63 (27.70)	29.54 (32.92)	37.45 (37.73)	51.18 (45.68)	65.76 (54.27)	93.16 (74.85)	100.00 (90.00)	100.00 (90.00)
S.Em.±	0.79	0.79	0.71	0.69	0.64	1.00	0.71	0.79	0.61
CD at 5%	2.31	2.31	2.069	2.00	1.87	2.91	2.08	2.31	1.79

** Figures in parenthesis arc sine transformed value*

Table 3. Terminal disease incidence and fruit yield in development bio-intensive disease management of pomegranate wilt during 2014-2015

Sl. No	Treatment No.	Disease incidence (% wilting of branches)		Pooled Mean	Wilt (%) reduction over control	Yield (t/h)		Pooled Mean	Yield (t/h) % increase over control
		2014	2015			2014	2015		
1	T_1	29.98 (33.20)	34.51 (35.97) *	31.91 (34.37)	67.75	7.05	7.09	7.07	7.07
2	T_2	10.28 (18.70)	10.58 (18.98)	10.53 (18.94)	89.57	10.31	10.43	10.37	10.37
3	T_3	44.11 (41.62)	50.01 (45.01)	46.91 (43.24)	52.94	4.95	5.18	5.06	5.06
4	T_4	18.33 (25.35)	18.53 (25.50)	18.44 (25.40)	81.57	8.70	8.78	8.74	8.74
5	T_5	19.93 (26.51)	20.66 (27.03)	20.31 (26.74)	79.70	8.59	8.55	8.57	8.57
6	T_6	57.08 (49.07)	57.77 (49.48)	57.41 (49.27)	42.57	3.53	3.60	3.56	3.56
7	T_7	51.79 (46.03)	53.83 (47.20)	52.53 (46.42)	47.19	4.13	3.86	3.99	3.99
8	T_8	48.27 (44.01)	51.12 (45.64)	49.92 (44.98)	50.30	4.69	4.73	4.71	4.71
9	T_9	52.72 (46.56)	55.55 (48.19)	54.90 (47.80)	45.86	4.01	4.13	4.07	4.07
10	T_{10}	53.12 (46.79)	56.00 (48.45)	54.00 (47.29)	45.44	3.75	3.86	3.81	3.81

Contd…..

Sl. No	Treatment No.	Disease incidence (% wilting of branches)		Pooled Mean	Wilt (%) reduction over control	Yield (t/h)		Pooled Mean	Yield (t/h) % increase over control
		2014	2015			2014	2015		
11	T_{11}	42.71 (40.81)	45.34 (42.33)	44.51 (41.83)	55.97	5.33	5.36	5.34	5.34
12	T_{12}	39.53 (38.96)	41.72 (40.23)	41.42 (40.00)	59.37	5.55	5.66	5.61	5.61
13	T_{13}	36.55 (37.20)	40.53 (39.54)	38.81 (38.54)	61.46	6.04	6.19	6.11	6.11
14	T_{14}	32.71 (34.89)	38.57 (38.39)	31.91 (34.37)	64.36	6.53	6.68	6.60	6.60
15	T_{15}	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	-	0.00	0.00	0.00	-
	S. Em. ±	0.82	0.61	0.59		0.09	0.12	0.10	
	CD at 5%	2.35	1.79	1.70		0.28	0.35	0.29	

** Figures in parenthesis arc sine transformed value*

Table 4. Economics of development of bio-intensive disease management trial on pomegranate as influenced by wilt disease

Treatment	Mean Yield (t/h)	Cost of cultivation (Rs ha^{-1})	Treatment cost (Rs ha^{-1})	Total cost of cultivation (Rs ha^{-1})	Gross returns (Rs ha^{-1})	Net returns (Rs ha^{-1})	C:B
T_1	7.07	275703	35437	311140	636300	325160	1:2.04
T_2	10.37	275703	83700	359403	933300	573897	1:2.59
T_3	5.06	275703	15828	291531	456300	164769	1:1.56
T_4	8.74	275703	7087	282790	786600	503810	1:2.78
T_5	8.57	275703	25312	301015	771300	470285	1:2.56
T_6	3.56	275703	25312	301015	321300	20285	1:1.06
T_7	3.99	275703	38452	314155	360000	45845	1:1.14
T_8	4.71	275703	32625	308328	423900	115572	1:1.37
T_9	4.07	275703	22365	298068	366300	68232	1:1.22
T_{10}	3.81	275703	16537	292240	342900	50660	1:1.17
T_{11}	5.34	275703	28901	304604	481500	176896	1:1.58
T_{12}	5.61	275703	25987	301690	504900	203210	1:1.67
T_{13}	6.11	275703	61076	336779	550800	214021	1:1.63
T_{14}	6.60	275703	58162	333865	594900	261035	1:1.78
T_{15}	7.07	275703	0.00	275703	0.00	0.00	1:0.00

3.2.4 Pooled data (2014 and 2015)

The results indicated that the highest fruit yield was obtained in propiconazole (0.2%) at 10.37 t/ha followed by diamond (*T. viride*) (8.74 t/ha) and *T. harzianum* (Th-R) at 8.57 t/ha when compared to no fruit yield in the untreated control treatment.

3.3. Benefit-cost ratio

The economics of the development of a bio-intensive management strategy against pomegranate wilt trial was worked out by taking into consideration the total cost of cultivation, cost of treatment, and gross returns. The highest CB ratio (1: 2.78) was obtained in drenching of diamond (*T. viride*) alone. The next best treatment is propiconazole alone which recorded a CB ratio of 1: 2.59 and another treatment *T. harzianum* (Th-R) also showed little bit more CB ratio (1: 2.56) when compared to the rest of the treatments (Table 4).

4. DISCUSSION

In vitro, screening of fungicides and antagonists provides preliminary information regarding their efficacy against *C. fimbriata* and with a hope to utilize the promising bio-agents and fungicides for the management of pomegranate wilt under field conditions. The development of a bio-intensive disease management strategy is an approach, where all methods of management may be brought into operation to reduce pathogenic activities to a tolerance or permissible level, with chemicals applied only when absolutely necessary. Since present-day agriculture is aiming towards sustainable agriculture and organic farming, the use of chemicals is mostly discouraged.

Management of *C. fimbriata* through soil application of fungicides is difficult because of its broad host range as well as its worldwide distribution which precludes such a strategy. Once established in the soil, it is difficult to eliminate the pathogen. Management through chemical methods leads to ill effects like residual toxicity, environmental pollution, and fungicide resistance. Although soil application with fungicides is recommended to minimize the infection at early stages, it does not give complete protection. A single method of management may not be possible to control this disease effectively. Bio-agent antagonists to manage disease receive a lot of attention. Resistance-inducing rhizobacteria offer an excellent alternative in providing natural, effective, safe, persistent, and durable protection. Plants have endogenous defense mechanisms that can be induced in response to the pathogen and bio-agents. One classical biotic inducer is the plant growth-promoting bacterium, *Pseudomonas fluorescens* [2]. Thus, the concept of bio-intensive disease management becomes the talk of the day, so as to; create the least hazard to man and the environment and to permit maximum assistance to natural control.

Keeping this in mind, various workers have succeeded in this aspect [7, 8, 9, 10, 11, 12, 13]. With this objective, the present investigation was carried out using chemicals and bio-agents singly and in combination, to manage the wilt of pomegranate for two consecutive years. The results on pooled data indicated that propiconazole (0.2%), three drenching at 15-day intervals showed significantly superior disease control by recording the least disease incidence (10.53%) with the highest mean fruit yield of (10.37 t/ha) against untreated check with wilt (100.0%) and absolutely no fruit yield. This treatment was followed by three drenchings of Diamond (*T. viride*) (0.7 g/l) and *Trichoderma harzianum* (Th-R) (5g/l) which recorded wilt (18.44% and 20.31%, respectively) and fruit yield (8.74 t/ha and 8.57 t/ha). Apart from this, per cent reduction in disease incidence also indicated that three drenching of propiconazole (0.2%) alone showed the highest per cent reduction of 89.57 per cent, while it was comparatively less in drenching treatment with Diamond (*T. viride*) (81.57%) and *T. harzianum* (Th-R) (79.70%). *C. fimbriata* survives under adverse conditions as mycelia within the plant host or as thick-walled aleurioconidia in the soil or in plant host or debris. Aleurioconidia, because of the thick wall, is probably the most common fungal survival structure in soil and most initial infections arise from such inoculums. *C. fimbriata* survives in the soil long time. Thus, effective treatments like propiconazole fungicide, Diamond (*T. viride*) and *T. harzianum* (Th-R) are well-known as effective biological control agents for *C. fimbriata*. [8] triazoles such as tebuconazole, cyperconazole, propiconazole, difenoconazole and diniconazole provide excellent control some soil borne diseases including wilt. [13] soil application of *T. viride* NRCB1 significantly reduced the *Fusarium* wilt disease of banana (up to 80%) and increased the plant growth parameters. [14] dipping of suckers in the suspension of *P. fluorescens* (10^6 CFU/ml) or *T. viride* (10^6 CFU/ml) along with the application of 500 g wheat bran: saw dust inoculum (1:3) of the respective bio-control agent three months after planting, effectively reduced the *Fusarium* wilt of banana incidence and produced the highest yield.

The results of the present study are similar to the study conducted by Thangavelu Raman and Gopi Muthukathan [12] in field evaluation of *Pseudomonas putida, Bacillus cereus, Rhizobium* spp., and *Acromobacter* spp. five combinations of bacterial isolates in an area of heavy inoculum and using *cv*. Grand Naine indicated that all the bacterial combinations suppressed *Fusarium* wilt and increased the number of banana hands and bunch weight compared to untreated control. Further, Sharma *et al*. [4] reported that soil drenching of affected and adjacent healthy plants with carbendazim or propiconazole (0.2%) + chloropyriphos (0.2%) has resulted in effective wilt management. Somasekhara [15] screened various fungicides against *C. fimbriata* and reported that propiconazole, boric acid, and phosphoric acid were found effective against wilt pathogen. Shruthi *et al*. [10] reported that *Trichoderma* species are among the most promising biocontrol fungi against many fungal plant pathogens and isolated and screened native isolates of *Trichoderma* spp. against wilt disease of pomegranate caused by *C. fimbriata*. *Trichoderma* isolates are more effective and show excellent control of *C. fimbriata*, responsible for Pomegranate wilt. These isolates could be exploited for their volatile compound production mechanism. The three superior isolates will be promising biocontrol agents against wilt and plant growth promotion activity in pomegranate seedlings and Kishor *et al,* [9] also reported that there are certain fungal and bacterial candidates well efficient in controlling diseases, genus *Trichoderma* has occupied a prestigious position among them. It is capable of managing seed and soil-borne plant diseases.

5. CONCLUSIONS

In the development of bio-intensive disease management, Field evaluation over two years indicated that three drenchings of propiconazole (0.2%), Diamond (*T. viride*) (0.7 g/l) and *T. harzianum* (Th-R) (5g/l) at an interval of 15 days showed maximum disease control with higher mean fruit yields and cost-benefit ratios.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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