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Biointensive Integrated Disease Management of Seed Borne *Fusarium graminearum* in Wheat (*Triticum aestivum* L.)

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Thirty wheat (*Triticum aestivum* L.) seed samples were collected from different wheat growing areas of Tamil Nadu. The pathogen *Fusarium graminearum* Schwabe recorded the maximum seed-borne incidence (38.6%) in Wellington wheat seed sample. The *Trichoderma harzianum* and *Pseudomonas fluorescens* (Pf1) exhibited high level of inhibition of 65.83 and 62.22 per cent over control against *F. graminearum* under *in vitro* condition respectively. Among the 46 plant extracts, zimmu leaf extract recorded minimum (15%) germination of *F. graminearum* macroconidia under *in vitro* condition. Application of Zimmu leaf extract 0.2 per cent sprayed during anthesis stage effectively controlled the seed-borne *F. graminearum* spread under glass house condition. Zimmu leaf extract, zimmu leaf extract + *T. harzianum,* zimmu leaf extract + *P. fluorescens* and zimmu leaf extract + *T. harzianum* + *P. fluorescens* spray treatments were on par with each other to record the least incidence of 15.60 to 17.25 per cent *Fusarium* head scab in field conditions. Among the eight treatments, zimmu leaf extract sprayed alone and combined with antagonists, decreased seed borne incidence and increases grain yield in wheat.

Key words: *Fusarium graminearum, Pseudomomas, Trichoderma,* Zimmu leaf extract

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Wheat is one of the main staple food of man and is grown in almost all the temperate and subtropical regions of the world. Seed-borne mycoflora of wheat include *Fusarium moniliforme* Sheldon*, Alternaria alternata, Drechslera sorokiniana* (Sacc.) Subram. and Jain*, Fusarium avenaceum* (Fr.) Sacc.*, Fusarium graminearum* Schwabe (*Gibberella zeae* [(Schwein.) Petch]*, Fusarium nivale* (Fr.) Samuels and Hallet*, Fusarium culmorum* (W.G. Smith) Sacc.*, Fusarium equiseti* (Corda) Sacc.*, Fusarium sporotrichioides* Sherb*, Cladosporium herbarum* Link ex Gray and *Stemphylium botryosum* Wallr (Glazek, 1997). *Fusarium graminearum* causing ear blight and scab of wheat was reported from Arunachal Pradesh and Wellington, Tamil Nadu (Saharan *et al.,* 2004) . The disease incidence could reduce kernel set and grain weight, thereby causing significant yield losses from 30 per cent to 70 per cent under epidemic conditions (Miedaner, 1997). Several effective pesticides have been recommended for use against this pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards. Biological control has been explored as an additional or alternative means of managing *Fusarium* head blight. Thus, more emphasis was laid on the combined use of two or more strains of bacteria, which turned out to be more successful than either of them alone, as reported by several workers (Saravanakumar *et al.,* 2007).

Higher plants contain a wide spectrum of secondary substances viz., phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins and sterols. Such plant chemicals exploited for their different biological properties (Sandosskumar *et al.,* 2007). Biological control of plant pathogens using antagonistic microorganisms and plant products is a vital area of plant pathological research in the present day to avoid environmental pollution. With this background, the present study was carried out to determine the efficacy of leaf extract of ‘‘Zimmu’’ and biocontrol agents against seed borne pathogen *F. graminearum* in wheat.

Materials and Methods

Isolation of pathogen

Wheat seed samples were collected from Farmers' field and different locations of Tamil Nadu *viz*., Coimbatore, Salem, Coonoor and Wellington and Seed Testing Laboratories (Coimbatore and Salem). *F. graminearum* was isolated from the infected seed samples by using *Dry seed examination and Blotter method. The fungi were purified by single spore isolation method (Riker and Riker, 1936) and identified to species level based on synoptic keys by Nelson et al. (1983).*

Isolation of biocontrol agents

Rhizosphere soil samples were collected from the different locations of Tamil Nadu, India. The biological antagonists were isolated using serial dilution plate technique. The fungal antagonists

Trichoderma viride (Tv1), *Trichoderma harzianum* (Th1) and bacterial antagonists *Pseudomonas fluorescens* (Pf1) and *Bacillus subtilis* (Bs1) were obtained from the Culture Collection Centre, Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India.

Testing of microbial antagonism in vitro

T. harzianum, T. viride and *P. fluorescens* and *B. subtilis* isolates were tested for their antagonism against the isolate of *F. graminearum* by dual culture technique (Dennis and Webster, 1971). It was replicated thrice and a suitable control was also maintained. The mycelial growth of pathogen was measured 48 h after inoculation of bacterial antagonists. The results were expressed as per cent growth inhibition over control by using the following formula: I=C - Tx100/C; Where, I = Inhibition of fungal growth, C = Fungal growth in control and T = Fungal growth in the treatments.

Effect of plant extracts on the spore germination and mycelia growth of F. graminearum

Inhibition spore germination assay was carried out by the method of Montgomery and Moore (1938). The leaf samples of herbs were collected from the herbal garden at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore and used for extraction and preparation of plant extracts. The per cent spore germination was recorded at each 3 h interval and expressed as per cent inhibition over control. The screened plants extracts were further evolved by using methanol and ethanol solvent extracts. Totally three plant leaf extracts were tested against the seed-borne *Fusarium* sp. by poisoned food technique under different concentrations (0.1, 0.2 and 0.5%).

Preparation of zimmu 50 per cent EC formulation for field spray

An emulsifiable concentrate (EC) formulation of zimmu was developed using methanol solvent. The leaf extract of zimmu was formulated to 50 EC containing 50 per cent (v/v) zimmu extract, using an organic solvent (cyclohexanone) and an emulsifier (Tween-80) and stabilizer (epichlorohydrin) (Karthikeyan *et al.,* 2007).

Management of seed borne F. graminearum on wheat under glass house conditions

Ten seeds of wheat were sown in autoclaved potting mix consisting of two parts compost and one part of field soil in 20 cm diameter plastic pots. There were three replicate pots per treatment, arranged in complete randomized design (CRD). Treatments were, T_1 - *Trichoderma harzianum* @ 2%*;* T_2 - *Trichoderma viride* (Erode isolate) @ 2%; T_3 - *Trichoderma viride* (Dharmapuri isolate) @ 2%; T_4 - *Pseudomonas fluorescens* (Pf1) @ 2%; T_5 - *Bacillus subtilis* 1 @ 2%; T_6 - Zimmu leaf extract @ 0.2%; T_7 - Adhathoda leaf extract @ 0.2%; T_8 - Insulin plant leaf extract @ 0.2%; T_9 –Control. Inoculation with *F. graminearum* began six hour after the biocontrol inoculation and continued every evening for 10 days. The concentration of spores in the inoculum was approximately $2\times10_5$ spores/ml of the mixture of the virulent isolate of *F. graminearum.* The inoculum of biocontrol agents or *Fusarium* @ 2% was applied with a sprayer at about 7 ml per spike of wheat. Inoculation with bacterial antagonist began when the main spikes emerged from the boot and was repeated 10 days later. In wheat head blight was evaluated by the severity, the number of necrotic spikelets in each spike divided into the number of spikelets in each spike 21 days after inoculation. Each panicle was threshed and pooled to get equal amount of grain from each panicle to make a bulk sample. The threshed grain mould rating (TGMR) was recorded for wheat, grains as per the method of Mayee and Datar (1985) by spreading the bulk grain in the Petri dish with the help of magnifying lens (× 10) under proper lighting using the 1 to 5 scales.

1 - No mould visible
2 - 1-10 per cent of the grain surface moulded
3 - 11-25 per cent of the grain surface moulded
4 - 26-50 per cent of the grain surface moulded
5 - More than 50 per cent of the grain surface moulded

Per cent grain colonization by individual mould fungi on threshed grain was recorded based on their symptoms. The mold severities of entries were evaluated for the effective bicontrol agent and plant extract.

Eco-friendly management of seed borne Fusarium sp. in field

In order to find out the efficacy of biocontrol agents and zimmu extract on the management of seed borne *F. graminearum* from November, 2008 to March, 2009 with wheat (HW 3094 CoW (1)) obtained from the ICAR Wheat Regional research station, Wellington, Ooty, Tamil Nadu. The field trials were conducted at Wellington for the following treatments are *via.*, T_1 - *Trichoderma harzianum* @ 2%*;* T_2 - *Pseudomonas fluorescens* (Pf1) @ 2%; T_3 - Zimmu leaf extract @ 0.2%; T_4 - *T. harzianum* @ 2% + *P. fluorescens* (Pf1) @ 2%; T_5 - *T. harzianum* @ 2% + Zimmu leaf extract 50 EC @ 0.2%; T_6 - *P. fluorescens* (Pf1) @ 2% + Zimmu leaf extract 50 EC@ 0.2%; T_7 - *T. harzianum* @ 2% + *P. fluorescens* (Pf1) @ 2% + Zimmu leaf extract 50EC @ 0.2%; T_8 – Control. The experiment was conducted in the randomized block design with a plot size of 5 × 3 m, adopting the recommended dosage of fertilizers and spacing. Three replications were maintained. The first spray of treatments was given at 50 per cent flowering and second spray was given 15 days after first spray and third spray was given 10 days after second spray. At the maturity stage five ear heads at random were collected in each replication. The resultant seed infection was worked out and expressed in percentage. The grain yield and

disease incidence was calculated by the dry seed observation and blotter method as mentioned earlier in material and methods recorded.

Statistical analysis

The field trials data were analysed using the IRRISTAT version 92-1 program developed by the Biometrics Unit, International Rice Research Institute, the Philippines. The percent infection, growth and yield data were analysed independently by trial. The data were subjected to analysis of variance (ANOVA). The treatment means were compared by Duncan's multiple range test (DMRT) (Gomez and Gomez 1984).

Results and Discussion

Thirty wheat seed samples were collected from farmers fields and Seed Testing Laboratories located in different parts of Tamil Nadu. Many mycoflora were isolated, among the them, *F. graminearum* recorded the maximum (38.6 %) in Wellington seed sample *In vitro* antagonistic activity of *Trichoderma* sp. were screened results indicated that *T. harzianum* inhibited the mycelial growth of *F. graminearum* to an extent of 65.83 per cent over control (Table 1). The bacterial antagonist isolates *P. fluorescens* Pf1 and *Bacillus subtilis* Bs1 exhibited high level of inhibition against *F. graminearum. P. fluorescens* Pf1 recorded the maximum inhibition of 62.22 per cent over control, followed by the isolate *Bacillus subtilis* Bs1 (55.83%) (Table 2). The potential of *Trichoderma* species as biocontrol agents against various plant diseases has been reported by several workers (Sharon *et al.,* 2001). Shanmugam *et al*. (2001) reported that the isolate Pf1 of *P. fluorescens* was the most effective in inhibiting the mycelial growth of *M. phaseolina in vitro* by 82.60 per cent.

Table 1. Effect of fungal antagonists on mycelial growth of *F. graminearum in vitro*

Fungal antagonist	Location	Isolate No.	Inhibition zone (mm)	Mycelial growth (mm) at 5 DAI*	Inhibition over control (%)
Trichoderma sp.	Bhavanisagar	FA 1	10.20	39.00	56.64 (48.82)
T. viride	Coimbatore	FA 2	8.85	45.75	49.17 (44.53)
Trichoderma sp.	Coonoor	FA 3	8.50	47.50	47.22 (43.41)
Trichoderma sp.	Dharmapuri	FA 4	10.50	37.50	58.33 (49.80)
Trichoderma sp.	Erode	FA 5	11.15	34.25	61.94 (51.91)
Trichoderma sp.	Ooty 1	FA 6	8.70	46.50	48.33 (44.04)
Trichoderma sp.	Ooty 2	FA 7	9.35	43.25	51.94 (46.11)
Trichoderma sp.	Ooty 3	FA 8	8.20	49.00	45.56 (42.45)
Trichoderma sp.	Paramathyvellur	FA 9	7.86	50.70	43.61 (41.33)
Trichoderma sp.	Pollachi	FA 10	9.90	40.50	55.00 (47.87)
Trichoderma sp.	Thanjavur	FA11	7.20	54.00	40.00 (39.23)
T. viride 1	TNAU, Coimbatore	FA 12	9.70	41.50	53.89 (47.23)
Trichoderma sp.	Thondamuthur	FA 13	8.60	47.00	47.78 (43.73)
T. harzianum	TNAU, Coimbatore	FA 14	11.85	30.75	65.83 (54.23)
Control	-		0.00	90.00	00.00 (00.72)
CD (P=0.05)			0.48		2.42

* Mean of three replications. DAI – Days after inoculation. Figures in the parentheses are arc sine transformed values;

Table 2. Effect of bacterial antagonists on mycelial growth of *F. graminearum in vitro* (Dual culture technique)

Bacterial Antagonist	Location	Isolate No.	Inhibition zone (mm)	Mycelial growth (mm) at 5 DAI*	% Inhibition over control
P. fluorescens	Coimbatore	BA 1	8.75	46.25	48.62 (44.21)
P. fluorescens	Ooty	BA 2	8.75	46.25	48.62 (44.21)
P. fluorescens (Pf1)	TNAU, Coimbatore	BA 3	11.20	34.00	62.22 (52.07)
P. fluorescens	Thondamuthur	BA 4	9.85	40.75	54.72 (47.71)
B. subtillis(Bs 1)	TNAU, Coimbatore	BA 5	10.05	39.75	55.83 (48.35)
B. subtillis	Coimbatore	BA 6	8.80	46.00	48.89 (44.37)
B. subtillis	Ooty	BA 7	9.00	45.00	50.00 (45.00)
Control	-	BA 1	0.00	90.00	0.00 (1.01)
CD (P=0.05)			0.51		2.65

* Mean of three replications. Figures in the parentheses are arc sine transformed values

Among the Forty- six plant extracts, the zimmu leaf extract recorded minimum (15%) germination of *F. graminearum* macroconidia, followed by Insulin plant and Adhatoda leaf extracts with 17 and 24 per cent spore germination (Table 3) . Among the four screened plant extracts, 0.2 per cent zimmu leaf extract recorded maximum inhibition of *F. graminearum* mycelial growth, both ethanol and methanol extracts. Between the solvents methanol extract recorded maximum inhibition (77.22%) over control. The similar studies were carried by Shekhawat and Prasada (1971). The extract of zimmu (*A. sativum* × *A. cepa)* at 0.5 per cent concentration exhibited strong antifungal activity against *A. niger, F. moniliforme, A. flavus, C. lunata* and *H. halodes* (Vasanth Baskar, 2007).

Table 3. Screening of plant leaf extracts against *F. graminearum* spore germination *in vitro*

Plant	Scientific Name	Spore germination (per cent)*			
		3 h	6 h	9 h	12 h
Adhatoda	*Adhatoda vasica*	2 (08.13)	6 (14.18)	12 (20.27)	24 (29.33)
Agapana toa	*Eupatorium triplinarve*	17 (24.35)	66 (54.33)	78 (62.03)	97 (80.03)
Agave	*Agave angustifoli*	12 (20.27)	41(39.82)	52 (46.15)	92 (73.57)
Aloe vera	*Aloe vera*	20 (26.57)	86 (68.03)	91 (72.54)	100 (89.79)
Alternanthera	*Alternanthera paronychioides*	22 (27.97)	92 (73.57)	98 (81.87)	100 (89.79)
Aswagandha	*Withania somnifera*	21 (27.28)	90 (71.57)	94 (75.82)	100 (89.79)
Blepharis	*Blepharis maderaspatensis*	21 (27.28)	89 (70.63)	98 (81.87)	100 (89.79)
Brahmi	*Bacopa monnieri*	17 (24.35)	78 (62.03)	91 (72.54)	100 (89.79)
Cissus	*Cissus quadrangularis*	18 (25.10)	73 (58.70)	79 (62.73)	97(80.03)
Coleus	*Coleus aromaticus*	19 (25.84)	76 (60.67)	90 (71.57)	98 (81.87)
Coleus	*Coleus forsholii*	27 (31.31)	91 (72.54)	96 (78.46)	100 (89.79)
Curry leaf	*Murraya koenigii*	16 (23.57)	78 (62.03)	92 (73.57)	100 (89.79)
Gymnema	*Gymnema sylvestre*	20 (26.56)	83 (65.65)	94 (75.82)	100 (89.79)
Henna	*Lawsonia inermis*	2 (08.13)	6 (14.18)	11 (19.37)	29 (32.58)
Hibiscus	*Hibiscus rosa sinensis*	20 (26.57)	79 (62.73)	91 (72.54)	99 (84.26)
Indian ipecac	*Abrus precatorius*	18 (25.10)	79 (62.72)	92 (73.57)	100 (89.79)
Insulin plant	*Costus speciosus*	0 (00.21)	6 (14.18)	12 (20.27)	17 (24.35)
Milk weed	*Calotropis gigantea*	22 (27.97)	89 (70.63)	98 (81.87)	100 (89.79)
Kallimudaiyan	*Caralluma* sp.	21 (27.28)	84 (66.42)	94 (75.82)	99 (84.26)
Karbokarasi	*Psoralea corylifolia*	22 (27.97)	90 (71.57)	96 (78.47)	100 (89.79)
Kayyantakarai	*Weddia chinensis*	20 (26.57)	91 (72.54)	96 (78.46)	100 (89.79)
Kesavarthini	*Serianthes amarantharides*	18 (25.10)	77 (61.34)	89 (70.63)	98 (81.87)
Kodikkalli	*Sarcostemma acidum*	21 (27.28)	86 (68.03)	91 (72.54)	100 (89.79)
Koriveli	*Plumbago zeylanica*	29 (32.58)	91(72.54)	94 (75.83)	99 (84.26)
Lemon grass	*Cympopogan flexuosus*	21 (27.28)	92 (73.57)	97 (80.03)	100 (89.79)
Lippia	*Lippia nodiflora*	21 (27.28)	91 (72.54)	97 (80.03)	100 (89.79)
Masipathiri	*Artemisia absinthium*	20 (26.57)	86 (68.03)	93 (74.66)	99 (84.26)
Nagamalli	*Rhinocanthus nausta*	3 (09.97)	10 (18.44)	48 (43.85)	85 (67.22)
Nagathanthi	*Baliospermum montanum*	19 (25.84)	82 (64.90)	96 (78.47)	100 (89.79)
Nirmulli	*Tygrophila auriculata*	20 (26.57)	86 (68.03)	91 (72.54)	99 (84.26)
Ocimum	*Ocimum tenuiflorum*	21 (27.28)	85 (67.22)	98 (81.87)	100 (89.79)
Sacred Basil	*Ocimum sanctum*	23 (28.67)	85 (67.22)	97 (80.03)	100 (89.79)

*Mean of three replications. Figures in the parentheses are arc sine transformed values

The selected antagonists and leaf extracts were tested under pot culture condition for their effectiveness individually on wheat seed -borne *Fusarium* sp. incidence (Table 4). Zimmu leaf extract

Table 4. Effect of antagonists and plant leaf extracts application on *Fusarium graminearum* incidence in wheat under pot culture

Treatment	Germination (%)	Per cent infected ear head	Percent disease reduction over control
Trichoderma harzianum	93.33	53.33	33.34
Trichoderma viride (Erode isolate)	86.76	66.67	16.65
Trichoderma viride (Dharmapuri isolate)	93.33	66.67	16.65
Pseudomonas fluorescens (Pf1)	93.33	40.00	50.00
Bacillus subtilis 1	93.33	60.00	25.00
Zimmu leaf extract	93.33	33.33	58.34
Adhathoda leaf extract	100.00	60.00	25.00
Insulin plant leaf extract	93.33	73.33	8.34
Control	93.33	80.00	-
CD (P=0.05)	5.23		

*Mean of three replications

0.2 per cent spray during anthesis stage effectively controlled the seed-borne *F. graminearum* spread under glass house condition. This treatment recorded the incidence of 33.33 per cent and also it showed minimum disease incidence in ear head. This was followed by *P. fluorescens* 1 (Pf1) 2 per cent spray which recorded the incidence of 40.00 per cent. Among the fungal antagonists *T. harzianum* recorded minimum incidence (53.33 per cent) of *Fusarium* head blight. The inhibitory effect of *Pseudomonas fluorescens* and *Bacillus subtilis* against *F. oxysporum* f. sp. *lycopersici* under *in vitro* condition has been reported by several workers (Sarath Chandra *et al.,* 1993).

Field trials were conducted during November 2008 to March 2009 with the effective treatments culled out from the previous pot culture experiments both at Wellington with seven treatments and a control. Among the treatments, T_3 T_5 T_6 and T_7 were on par with each other to record the least incidence of 15.60 to 17.25 per cent Fusarium head scab in Wellington trial. This was followed by combined application of *T. harzianum* and *P. fluorescens* with 20.50 per cent of scab incidence (Table 5). Among the different treatments, zimmu leaf extract (50% EC formulation) either alone or in combination was found effective. Karthikeyan *et al*. (2007) reported that foliar application of 50 EC formulation of zimmu at 0.3 per cent on 60, 75 and 90 days after sowing effectively reduced the incidence of grain mould upto 70 per cent. Sathya et al. (2005) reported that among the plant extracts tested, the leaf extract of Zimmu and snake wood (Strychnos nuxvomica Linn.) showed the highest antifungal activity against Rhizoctonia solani. *T. harzianum* has been proved effective against several soil and seed borne diseases (Poddar *et al.,* 2004). The maximum grain yield was observed in combined application of

Table 5. Efficacy of antagonists and plant leaf extracts on *F. graminearum* incidence under field conditions in wheat

Treatment	Germination (%)	Disease Incidence (%)	Percent disease Reduction over control	Seed yield (kg/ha)
T. harzianum @ 2%	93.50	31.75	17.68	3859.0
P. fluorescens(Pf1) @ 2%	95.00	30.00	22.22	3865.0
Zimmu leaf extract @ 0.2%	94.50	17.25	55.28	4034.0
T. harzianum @ 2% + *P. fluorescens* @ 2%	93.00	20.50	46.85	3871.0
T. harzianum @ 2%+ Zimmu leaf extract@ 0.2%	93.50	16.80	56.44	4040.0
P. fluorescens (Pf1) @ 2% + Zimmu leaf extract@ 0.2%	94.00	16.50	57.22	4045.0
T. harzianum @ 2% + *P. fluorescens* (Pf1) @ 2% + Zimmu leaf extract @ 0.2%	94.50	15.60	59.55	4063.0
Control (Untreated)	95.00	38.57	—	3780.0
CD (P=0.05)	8.15	2.15		133.28

*Mean of three replications

zimmu leaf extract, *T. harzianum* and *P. fluorescens* (T_7) treatment, which recorded 4063 kg/ha of wheat grains. This was followed by zimmu leaf extract combined with *P. fluorescens* (T_6) with 4045 kg/ha of wheat grains. *P. fluorescens, T. viride* and *T. harzianum* enhanced the germination in the mould infected seeds and the effect was superior to chemical seed treatments (Indira *et al.,* 2004). Vivekananthan *et al.* (2004) reported that application of fluorescent pseudomonads increased the fruit yield in mango. Saravanakumar *et al.* (2007) demonstrated the plant growth-promoting activity of *P. fluorescens* strains under field conditions. The present study concluded that application of biocontrol agents singly and in combination with Zimmu leaf extract was found to be effective in controlling the seed borne pathogen F. graminearum under pot and field conditions. Thus plant products and bioagents can be well exploited in the future and active principles from the plant extracts can also be isolated and formulated for the effective management of various plant diseases.

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