academicJournals

Vol. 10(40), pp. 1711-1714, 28 October, 2016
DOI: 10.5897/AJMR2016.8026
Article Number: 4E0277261342
ISSN 1996-0808
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African Journal of Microbiology Research

Full Length Research Paper

Efficacy of biocontrol agents in the management of head rot of cabbage (*Brassica oleracea var. capitata*) caused by *Sclerotinia sclerotiorum*

Kamesh Krishnamoorthy K.*, Sankaralingam A. and Nakkeeran S.

Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore-641003, India.

Received 24 March, 2016; Accepted 26 April, 2016

Head rot of cabbage caused by *Sclerotinia sclerotiorum* leads to rotting of fully matured cabbage heads in the field. In the present study the antagonistic effects of twenty *Bacillus* isolates was tested against *S. sclerotiorum in vitro*. Eight effective *Bacillus* isolates obtained from studies *in vitro*, commercial formulations of *Trichoderma viride* isolate TV-1 and *Pseudomonas fluoroscens* isolate Pf-1along with a fungicide check (Nativo-Tebuconazole+Trifloxystrobin) were carried further for field studies. Results of field studies indicated that fungicide check of Nativo (1.5 g/L) was highly effective with least disease incidence of 10.36% indicating 74.50% reduction over control. Among the biocontrol agents commercial formulation of *Trichoderma viride* isolate TV-1 was the most effective showing disease incidence of 11.38% indicating 72.00% reduction over control followed by *Bacillus amyloliquefaciens* isolate B15 and *Pseudomonas fluoroscens* isolate Pf-1 showing disease incidence of 13.24 and 13.31% indicating 67.41 and 67.24% reduction over control respectively and both treatments were on par. *B. licheniformis* isolate B16 was found to be least effective with 20.41 percent disease incidence indicating 49.76% reduction over control.

Key words: Bacillus, commercial formulation, fungicide check, *Pseudomonas fluoroscens, Trichoderma viride, Sclerotinia sclerotiorum.*

INTRODUCTION

Head rot of cabbage caused by the pathogen *Sclerotinia sclerotiorum* leads to rotting of fully grown cabbage heads in the field and during post-harvest operations and storage (Hudyncia et al., 2000). The pathogen is geographically cosmopolitan and has a broad ecological distribution (Purdy, 1979). The broad host range of the pathogen includes high value crops like alfala, bean, cabbage, canola, lettuce, peanut, soybean, sugarbeet, sunflower, tobacco and tomato (Grau, 1988; Farr et al., 1989).

The relatively unreliable control of *S. sclerotiorum* with traditional methods and concerns about pesticide residues has prompted interest in biological control as an alternative disease management strategy (Fernando et

*Corresponding author. E-mail: kamukrishagri@gmail.com. Tel: - 9944705207.

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al., 2007). Biological control of S*. sclerotiorum* has received considerable attention as an alternative disease management tactic to the use of fungicides due to its ability to provide safe and environmentally friendly disease control (Xiaojia et al., 2013).

Attributes of *Bacillus* spp. such as high thermal tolerance and ready formulation of endospores makes it an ideal agent for the development of commercial products. They adhere firmly to root surface especially when an inoculum of spore is used (Shoda, 2000). *Bacillus* spp. are known to survive well under field conditions due to the production of endospores (Boyetchko et al., 1999; Collins and Jacobsen, 2003). *Bacillus* spp. isolated from rhizosphere of common bean were tested for their antifungal activities against *S. sclerotiorum*. The maximum inhibition in radial growth caused by *Bacillus* spp.BPR7 was observed after seven days of incubation in dual culture and cell free culture filtrate (Pankaj et al., 2012). Fernando et al. (2013) reported that the metabolites produced by *B. subtilis* were antagonistic to the fungus with 17.7% reduction in mycelial growth which was constant even after two weeks suggesting high efficiency of the metabolites in control of *S. sclerotiorum*.

The aim of the present study was to determine efficacy of biocontrol agents under *in vitro* and field conditions.

MATERIALS AND METHODS

Screening of *Bacillus* isolates *in vitro*

Standard isolates of *Bacillus* spp. maintained as glycerol stocks were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The isolates were subcultured and maintained on Nutrient agar (NA) medium for further studies. The antagonistic effects of twenty *Bacillus isolates* were tested against *S. sclerotirum* by dual culture technique. A 9-mm-dia mycelial disc of the pathogen was placed at one end of the Petri plate containing combination medium (Potato Dextrose Agar + Nutrient Agar) and the bacterial antagonist was streaked at the opposite end. Inoculation of the pathogen without antagonist served as control and each treatment was replicated three times. The plates were kept in an incubator at 20±2°C. When the fungus attained full growth in the control plate, growth of the pathogen and inhibition zone were measured and percent reduction in growth over control was calculated.

Percent inhibition over control was calculated using the formula:

$$\frac{C - T}{T} \times 100$$

Where C = growth of *S. sclerotiorum* in control; T = growth of *S. sclerotiorum* in treatment.

Commercial formulations of *Trichoderma viride* and *Pseudomonas fluoroscens*

The commercial formulations of *Trichoderma viride* (TV-1) as talc formulation and *Pseudomonas fluoroscens* (Pf-1) as liquid formulation were obtained from *Trichoderma* Lab and *Pseudomonas* Lab respectively from Department of Plant Pathology, Centre for Plant Protection Studies (CPPS), Tamil Nadu Agricultural University , Coimbatore, Tamil Nadu, India.

Field trial

Eight effective *Bacillus* isolates obtained from *in vitro* studies, commercial formulations of *Trichoderma viride* (TV-1) and *Pseudomonas fluoroscens* (Pf-1) along with a fungicide check (Nativo-Tebuconazole+Trifloxystrobin) were carried further for field studies.

The field trial was conducted between February and March 2014 in the Kothagiri area of Nilgiris district and was laid out in a randomised block design. First spraying was done 48 days after planting when the cabbage was at cupping stage and subsequent sprayings were done at 7 days interval till head fill stage of the cabbage.

There were nine treatments in all consisting of *Bacillus amyloliquefaciens* (B15), *B. licheniformis* (B 19), *B. cereus (*B31), *B. licheniformis (*BSD-1), *B. subtilis* (B14), *B. licheniformis (*B16), *Pseudomonas fluorescens* (Pf-1) (commercial formulation), *Trichoderma viride* (TV1) (commercial formulation) each at a concentration of 10 mL/L and Fungicide check- Nativo (Tebuconazole+Trifloxystrobin) at a concentration of 1.5 g/L. The control had no bio control agent treatment at all.

The percent disease incidence was calculated using the following formula:

$$\frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Results were expressed in terms of per cent disease reduction over control which was calculated as follows:

$$R = \frac{100\ (C\text{-}T)}{C}$$

Where, R = Percent reduction over control; C = Percent disease incidence in control; T = Percent disease incidence in treatment.

Data analysis method

Statistical analysis was performed using the IRRISTAT software version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Before performing the statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were separated by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Twenty *Bacillus* isolates were tested *in vitro* against *S. sclerotiorum*. The treatment B15 (*B. amyloliquefaciens*) yielded the minimum mycelial growth of 53.0 mm and the highest growth reduction of 41.0% with an inhibition zone of 26.6 mm (Table 1). This was followed by B19 (*B. licheniformis*) which recorded 54.3 mm growth with39.7% growth reduction with an inhibition zone of 24.3 mm. However both the treatments were at par with each other.

Table 1. Effect of *Bacillus* isolates on the growth of *S. sclerotiorum* under *in vitro.*

Isolates	Mycelial growth (mm)	Inhibition over control (%)	Inhibition zone (mm)
B31-*Bacillus cereus*	55.0^{a}	39.0	23.6^{a}(4.90)
B14-*B.subtilis*	62.3^{bc}	30.7	17.3^{b}(4.22)
B19-*B.licheniformis*	54.3^{a}	39.7	24.3^{a}(4.98)
B12-*B.licheniformis*	65.3^{cd}	27.7	14.6^{bc}(3.89)
BSC-2-*B.tequilensis*	71.0^{ef}	21.0	0.0^{f} (0.70)
BSC-3-*B.subtilis*	73.0^{efg}	19.0	0.0^{f}(0.70)
BSC-6-*B.amyloliquefaciens*	74.7^{fg}	17.0	0.0^{f}(0.70)
BSC-7-*B.amyloliquefaciens*	65.0^{cd}	27.7	7.13^{d}(2.76)
B18-*B.megaterium*	73.3^{efg}	18.8	0.0^{f}(0.70)
B15-*B.amyloliquefaciens*	53.0^{a}	41.1	26.6^{a}(5.21)
B16-*B.licheniformis*	62.7^{bc}	30.3	6.6^{d}(2.67)
B30-*B.subtilis*	69.0^{de}	23.3	8.57^{d}(3.01)
B2-*B.amyloliquefaciens*	74.0^{fg}	17.7	0.0^{f}(0.70)
Bag3-*B.megaterium*	76.7^{g}	14.7	0.0^{f}(0.70)
B11-*B.licheniformis*	87.7^{h}	2.3	0.0^{f}(0.70)
B17-*B.megaterium*	72.0^{efg}	20.0	3.50^{e}(2.00)
BSc-9-*B.amyloliquefaciens*	76.0^{g}	15.7	0.0^{f}(0.70)
BSD-1-*B.licheniformis*	60.0^{b}	33.3	11.7^{c}(3.50)
B3-*B.subtilis*	74.3^{fg}	17.7	0.0^{f}(0.70)
BSC-5-*B.cereus*	72.3^{efg}	19.7	0.0^{f}(0.70)
Control	90.0^{h}	-	0.0^{f}(0.70)

[1]Figures in parenthesis are square root transformed values; [2]In a column means followed by same letter are not significantly different at the 5% level of DMRT.

Table 2. Efficacy of biocontrol agents under field conditions for the management of *Sclerotinia* head rots of cabbage.

T. No.	Treatments	Disease incidence (%)	Reduction over control (%)	Yield (t/ha)
T_1	B15-*Bacillus amyloliquefaciens* at 10ml/L	13.24^{c}(21.34)	67.41	44.68^{c}
T_2	B19- *B. licheniformis* at10ml/L	15.50^{d}(23.18)	61.85	41.64^{d}
T_3	B31- *B. cereus* at10ml/L	16.50^{e}(23.96)	59.38	40.71^{e}
T_4	BSD-1-*B. licheniformis*at10ml/L	18.57^{f}(25.52)	54.29	40.69^{e}
T_5	B14-*B. subtilis*at10ml/L	19.10^{g}(25.91)	53.00	40.44^{f}
T_6	B16-*B. licheniformis*at10ml/L	20.41^{h}(26.85)	49.76	40.31^{g}
T_7	Pf1-*Pseudomonas fluorescens* (commercial formulation) at 10 ml/L	13.31^{c}(21.39)	67.24	44.56^{c}
T_8	TV-1-*Trichoderma viride* (commercial formulation) @ 10g/L	11.38^{b}(19.71)	72.00	46.34^{b}
T_9	Fungicide check -Nativo (Tebuconazole+Trifloxystrobin)@1.5g/L	10.36^{a}(18.77)	74.50	47.86^{a}
T_{10}	Control	40.63^{i}(39.60)	-	37.45^{h}

Figures in paranthesis are arc sine transformed values; In a column means followed by same letter are not significantly different at the 5% level of DMRT.

B. amyloliquefaciens inhibited growth of *S. sclerotiorum in vitro* which was indicated by an inhibition zone between the two organisms (Abdullah et al., 2008). Strains of *B. amyloliquefaciens* ARP_23 and MEP_218 caused alterations in sclerotial morphology and sclerotial germination of *S. sclerotiorum* causing stem rot of soybean according to Alvarez et al. (2012). Under *in vitro* *B. licheniformis* strain 9555 showed effective antifungal activity against *S. sclerotiorum* (Vipin et al., 2012).

Results of field studies indicated that fungicide check of nativo applied at a concentration of 1.5 g/L was highly effective with least disease incidence of 10.36% indicating 74.50% reduction over control (Table 2). Among the biocontrol agents commercial formulation of

T. viride isolate (TV-1) was the most effective treatment showing disease incidence of 11.38% indicating 72.00% reduction over control. This was followed by *B. amyloliquefaciens* isolate (B15) and *P. fluorescens* isolate (Pf-1) showing disease incidence of 13.24 and 13.31% indicating 67.41 and 67.24% reduction over control respectively and both treatments showed no significant difference. *B. licheniformis* isolate (B16) was found to be least effective with 20.41% disease incidence indicating 49.76% reduction over control. Maximum yield of 47.86 t/ha was observed for the fungicide check - Nativo treatment (Table 2). This was followed by the *T. viridae* (TV-1) treatment with 46.38 t/ha yield. Gaur et al. (2010) reported that under field conditions *Sclerotinia* stem rot of mustard caused by *S. sclerotiorum* was effectively controlled by seed treatment (10g/kg) and foliar spray (0.2 per cent) at 50 days after sowing with talc based mixed formulation of *T. hamatum* and *T. viridae* in the ratio of 1:1 followed by bioagent combination of *T. harzianum* (10 g/kg) and *Gliocladium virens* (0.2%) over two consecutive years. *P. fluorescens* isolate (P13) decreased severity of *Sclerotinia* stem rot of oilseed rape by 59% under field conditions and also promoted seedling growth (Li et al., 2011). Use of *B. amyloliquefaciens* as a soil treatment supressed stems rot of cucumber caused by *S. sclerotiorum* (Sharie et al., 2013).

Conflict of Interests

The authors have not declared any conflict of interests.

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