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Compatibility between fungicides and *Bacillus amyloliquefaciens* isolate B15 used in the management of *Sclerotinia sclerotiorum* causing head rot of cabbage

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Abstract
Sclerotinia sclerotiorum is a necrotrophic plant pathogen. It is soil borne and affects temperate crops. The pathogen infects cabbage leading to a diseased condition known as head rot in which rotting of fully grown cabbage heads are observed. The rotted cabbage heads exhibit cottony white mycelial growth on their surface. With advancement of the disease the mycelial growth becomes dense and numerous carbon black coloured bodies called sclerotia are formed on the surface. Fungicides and *Bacillus amyloliquefaciens* isolate B15 used for the management of *S. sclerotiorum* were used for compatibility studies. Compatibility between eight fungicides *viz*., propineb, carbendazim, tebuconazole, Nativo (Tebuconazole + Trifloxystrobin), fosetyl aluminium, tricyclazole, metalaxyl, kresoxim methyl and *Bacillus amyloliquefaciens* isolate B15 was tested by poisoned food technique. Out of eight fungicides, compatibility between two effective fungicides *viz*., Nativo (Tebuconazole + Trifloxystrobin) and Carbendazim with *B. amyloliquefaciens* isolate B15 was tested by turbidometric method. Results of poisoned food technique indicated that *B. amyloliquefaciens* isolate B15 was compatible with Kresoxim methyl and Carbendazim at all the tested concentration. In turbidometric method fungicides nativo and carbendazim were compatible with *B. amyloliquefaciens* isolate B15 at 25, 50 and 100 ppm. The compatibility test can be used as a guide for combined application of fungicides and biocontrol agents in field level.

Keywords: *Sclerotinia sclerotiorum*, Fungicides, *Bacillus amyloliquefaciens*, Poisoned food technique, Turbidometric method

1. Introduction
Head rot of cabbage caused by *S. sclerotiorum* causes watery rot of fully grown cabbage heads in the field, during post-harvest operations and storage (Hudyncia *et al.*, 2000). Sclerotia are the primary survival structures of the pathogen (Korf and Dumont, 1972) [18]. Sclerotia formed on or within the host tissue are dislodged on the soil surface by wind or during harvesting and threshing (Cook *et al.*, 1975) [7]. Approximately 90 per cent of the life cycle of *Sclerotinia* species is spent in soil as sclerotia (Adams and Ayers, 1979) [1]. Sclerotia are hard asexual resting structures composed of vegetative hyphal cells which become interwoven and aggregate together.

In India head rot of cabbage was first reported during Febraury 1978 from Kodaikanal area in Dindigul district of Tamil Nadu (Alagianagalingam *et al.*, 1978) [3]. Infection of cabbage can occur when infected materials such as neighbouring cabbage plants, fallen flower petals, or pollen contact with healthy tissue (Mc Lean, 1949; Mc Lean 1958; Dillard *et al.*, 1986) [19, 20, 10]. Long distance transmission potential of *Sclerotinia* spp. is most likely by seed infected with mycelia, or sclerotia mixed with the seed (Blodgett *et al.*, 1946; Adams and Ayers, 1979) [4, 1].

In addition to physiological mechanisms of disease spread the pathogen can spread between diseased and healthy plants by transportation of infected pollen grains (Stelfox *et al.*, 1978) [25] and by direct contact (Huang and Hoes, 1980) [14]. On cabbage the symptoms first appear as water soaked spots on lower or upper leaves which enlarge causing the infected tissue to become soft followed by wilting of outer leaves. As the disease progresses a white cottony growth becomes evident on the leaves which finally covers the entire head followed by the development of sclerotia (Purdy, 1979) [22] *S. sclerotiorum* attacks nearly all kinds of succulent plants including flowers, shrubs, weeds and almost all vegetables (Chupp and Sherf, 1960) [6].

The pathogen has a broad host range which includes high value crops like alfalfa, bean, cabbage, canola, lettuce, peanut, soybean, sugarbeet, sunflower, tobacco and tomato (Grau, 1988; Farr *et al*., 1989) [11].

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 al.,* 2007) [12]. 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) [28]. Biological control agents derived from antagonistic fungi and bacteria had effectively reduced sclerotia of *S. sclerotiorum* in many cropping systems such as lettuce, celery (Budge and Whipps, 1991; Chitrampalam *et al.,* 2008) [5], soybean (Del Rio *et al.,* 2002) [8] and dry bean (Huang *et al.,* 2000) [15].

Vidhya *et al*. (2012) [27] tested compatibility between six strains of *Bacillus* and four fungicides. All the six strains were compatible with carbendazim showing a +++ (good growth) category growth in all dosages followed by hexaconazole. Growth of *Bacillus* was absent in media amended with copper oxychloride and mancozeb at all the dosages tested. Valarmathi *et al.* (2013) [26] tested compatibility between copper hydroxide and *B. subtilis* by turbidometric method and found that *B. subtilis* was compatible with copper hydroxide at a high concentration of 300 ppm. *B. subtilis* was highly compatible with azoxystrobin 25 SC even at the highest concentration of 250 ppm (Ahila *et al*., 2013) [2].

2. Materials and Methods

2.1. Selection of fungicides and biocontrol agent

Eight fungicides tested previously for the management of *Sclerotinia sclerotiorum* were used for the compatibility studies. Among twenty different *Bacillus* isolates screened previously against *S.sclerotiorum*, *B. amyloliquefacines* isolate B15 was the most effective one showing 41.1 per cent inhibition over control in dual plate assay under *in vitro* and 67.41 per cent disease reduction over control under field conditions (Kamesh *et al*., 2016) [16, 17]. Standard isolate of *B. amyloliquefaciens* isolate B15 maintained as glycerol stock was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The isolate was subcultured and maintained on Nutrient agar (NA) medium for further studies.

2.2. Study on compatibility between fungicides and effective bacterial antagonist

2.2.1. Poisoned food technique

Compatibility between eight fungicides *viz*., propineb, carbendazim, tebuconazole, Nativo (tebuconazole +trifloxystrobin), fosetyl aluminium, tricyclazole, metalaxyl, kresoxim methyl each at four concentrations (25ppm, 50ppm, 100ppm, 250 ppm) and *B. amyloliquefaciens* isolate B15 (effective isolate) were tested by poisoned food technique. Each treatment was replicated three times with proper control. The bacterial growth was observed after two days.

2.2.2. Turbidometric method

Among eight different fungicides screened against *S. sclerotiorum*, the compatibility between two effective fungicides viz., Nativo (tebuconazole+trifloxystrobin) and Carbendazin with *B. amyloliquefaciens* isolated B15 was tested by turbidometric method. Bacterial culture of *B. amyloliquefaciens* isolate B15 was transferred to 250 ml conical flasks containing 100 ml of Nutrient broth each and were amended with fungicides Nativo (tebuconazole + trifloxystrobin) and carbendazim at four different concentrations *viz*., 25, 50, 100 and 250 ppm. Flask containing bacterial culture alone was used as control. Each treatment was replicated three times. Lysis of the bacterial cells due to the fungicidal effect was measured as a decrease in turbidity of the solution which was determined through the optical density. The flasks were kept in a shaker and after 12 h the optical density values of the culture broth were determined in spectrophotometer at 610 nm at regular intervals of 6 h.

2.3. Statistical analysis

The data recorded were analysed statistically using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI), the Philippines (Gomez and Gomez, 1984). Data were subjected to analysis of variance (ANOVA) at two significant levels ($P< 0.05$ and $P< 0.01$) and means were seperated by Duncan'sMultiple Range Test (DMRT).

3. Result And Discussion

3.1 Compatibility between fungicides and effective bacterial antagonist

3.1.1 Poison food technique

Results of compatibility test showed that *B. amyloliquefaciens* isolate B15 was compatible with kresoxim methyl and carbendazim exhibiting good growth (+++) at concentrations of 25 and 50 ppm and moderate growth (++) at 100 and 250 ppm (Table 11; Plate 15). Propineb was not compatible with B15 at all the tested concentrations.

3.1.2 Turbidometric method

Fungicides carbendazim and Nativo (tebuconazole + trifloxystrobin) were highly compatible with B15 at 25, 50 and 100 ppm where turbidity was found to increase with incubation time upto 36h (Table 12 and 13; Fig 1 and 2). Compatibility of carbendazim with B15 was lowest at concentration of 250 ppm where decrease in turbidity was observed beyond 24h. Nativo (tebuconazole + trifloxystrobin) displayed low compatibility with B15 at concentration of 250 ppm where decrease in turbidity was observed beyond 30h. Both carbendazim and nativo displayed decrease in turbidity beyond 36h at all the concentration tested. In poisoned food technique nativo was not compatible with *B. amyloliqufaciens* isolate B15 at 250 ppm concentration.

Compatibility studies between effective fungicides Nativo (tebuconazole+trifloxystrobin) and carbendazim with *B. amyloliqufaciens* isolate B15 by turbidometric method revealed that both the fungicides were compatible with *B. amyloliqufaciens* isolate B15 at 25, 50 and 100ppm. Rajesh (2013) [23] tested compatibility between *B. cereus* isolate BSC5 and found that kresoxim methyl was compatible with BSC5 at 1000 and 1500 ppm showing moderate growth (++). Dheepa (2013) [9] reported that *B. cereus* isolate BSC1, *B. amyloliquefaciens* isolate BSC7, *B. licheniformis* isolate BSD1 and *B. megaterium* isolate BM were compatible with nativo at 1000 and 2000 ppm showing moderate growth. Mohiddin and Khan (2013) reported that *B. Subtilis* was compatible with carbendazim where the bacteria showed tolereance upto concentration of 50,000 µg/ml of the chemical. Sendhilvel *et al.* (2004) [24] reported that in

turbidometric method *B.subtilis* was compatible with azoxystrobin at 100, 150, 200, 250 and 300 ppm.

Table 1: Compatibility between fungicides and effective bacterial antagonist B15 (*Bacillus amyloliquefaciens*)- (Poisoned food technique)

S. No	Fungicide	Concentration			
		25	50	100	250
1	Nativo(Tebuconazole+Trifloxystrobin)	+++	++	++	-
2	Metalaxyl	+	+	-	-
3	Tricyclazole	+++	+++	++	-
4	Propineb	-	-	-	-
5	Tebuconazole	+++	+++	++	-
6	Fosetyl Al	++	+	-	-
7	Kresoxim methyl	+++	+++	++	++
8	Carbendazim	+++	+++	++	++
9	Control	+++	+++	+++	+++

(+++): Good growth (+): Poor growth
(++): Moderate Growth (-): No growth

Table 2: Compatibility of effective fungicide carbendazim with *Bacillus amyloliquefaciens* isolate B15 (Turbidometric method)

Time (hrs) After inoculation	Concentration (ppm) of carbendazim / OD value at 610 nm*				Control
	25	50	100	250	
12	1.8276^{g}	1.6628^{f}	1.2220^{e}	0.8518^{e}	1.9818^{g}
18	1.8636^{f}	1.7417^{e}	1.2354^{d}	0.9328^{d}	2.1274^{f}
24	2.0263^{d}	1.8813^{d}	1.3283^{c}	1.3861^{a}	2.3404^{e}
30	2.2168^{b}	2.0604^{c}	1.5328^{a}	1.3830^{a}	2.4887^{d}
36	2.3214^{a}	2.3412^{a}	1.5381^{a}	1.2219^{b}	2.7434^{c}
42	2.1808^{c}	2.2203^{b}	1.3651^{b}	1.0467^{c}	2.9792^{b}
48	1.9300^{e}	1.8688^{d}	1.0631^{f}	1.0417^{c}	3.1094^{a}

In a column means followed by same letter are not significantly different at the 5% level of DMRT

Table 3: Compatibility of effective fungicide nativo (tebuconazole + trifloxystrbin) with *Bacillus amyloliquefaciens* isolate B15 (Turbidometric method)

Time (hrs) After inoculation	Concentration (ppm) of native / OD value at 610 nm*				Control
	25	50	100	250	
12	1.8374^{d}	1.6041^{e}	1.2217^{c}	0.6340^{f}	1.9818^{g}
18	2.0838^{c}	1.6750^{d}	1.3394^{b}	0.7470^{d}	2.1274^{f}
24	2.1702^{b}	1.6921^{c}	1.3423^{b}	0.7870^{c}	2.3404^{e}
30	2.2313^{b}	1.8068^{b}	1.3500^{b}	0.8457^{a}	2.4887^{d}
36	2.3369^{a}	2.0337^{a}	1.4799^{a}	0.8170^{b}	2.7434^{c}
42	1.6049^{e}	1.5620^{f}	1.2403^{c}	0.6800^{e}	2.9792^{b}
48	1.2020^{f}	1.0339^{g}	0.9709^{d}	0.4555^{g}	3.1094^{a}

In a column means followed by same letter are not significantly different at the 5% level of DMRT

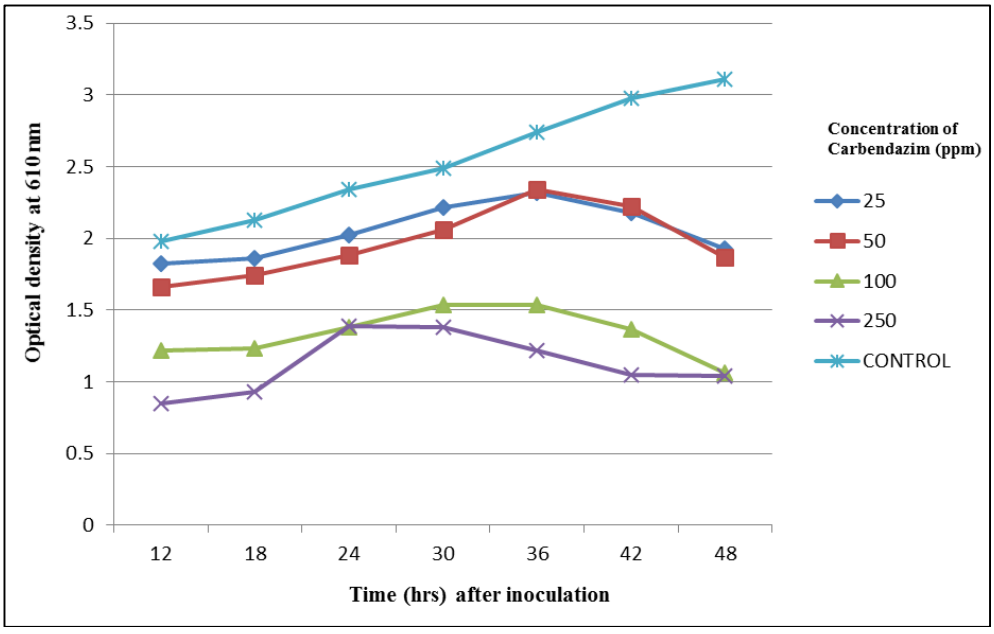

Fig 1: Compatibility of effective fungicide carbendazim with *Bacillus amyloliquefaciens* isolate B15

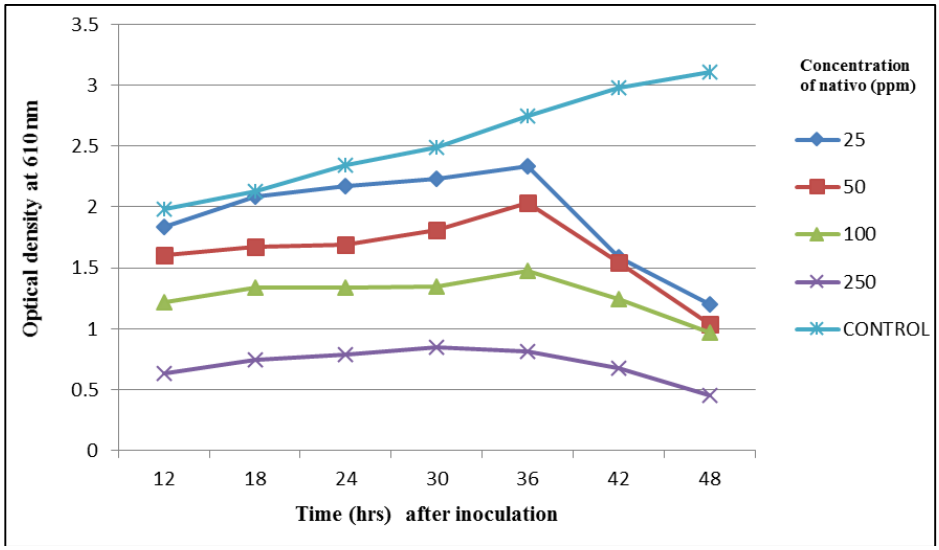

Fig 2: Compatibility of effective fungicide native (tebuconazole + trifloxystrbin) with *Bacillus amyloliquefaciens* isolate B15

References

1. Adams PB, Ayers WA. Ecology of *Sclerotinia* species. *Phytopathol.* 1979; 69:896-899.
2. Ahila DP, Prakasam V. Compatibility nature of Azoxystribin 25SC with *Pseudomonas fluorescens* and *Bacillus subtilis* on chilli plants. *W.J.A.S.* 2013; 18:258-264.
3. Alagianagalingam MN, Mohan R, Subramaniam CL, Sampathamoorthy S. A new record of *Sclerotinia* rot of cabbage in India. Curr. Sci. 1978; 47:967-968.
4. Blodgett EC. The *Sclerotinia* rot disease of beans in Idaho. *Plant Dis. Rep*., 1946; 30:137-139.
5. Chitrampalam P, Figuili PJ, Matheron ME, Subbarao KV, Pryor BM. Biocontrol of lettuce drop caused by *Sclerotinia sclerotiorum* and S.minor in desert agroecosystems. Plant Dis. 2008; 92:1625-1634.
6. Chupp C, Sherf AF. Sclerotinia diseases in Vegetable Diseases and their Control. The Ronald Press Company, New York. 1960, 43-46.
7. Cook GE, Steadman JR, Boosalis MG. Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans. Phytopathol. 1975; 65:250-255.
8. Del Rio LE, Martinson CA, Yang XB. Biological control of *Sclerotinia* stem rot of soybean with *Sporidesmium sclerotivorum*. Plant Dis. 2002; 86:999-1004.
9. Dheepa R. Biological and chemical control of white rust (*Puccinia horiana* Henn) and crown gall (*Agrobacterium tumefaciens* Smith and Townsend) of chrysanthemum under protected cultivation. M.Sc. (Agri.) Thesis, Tamil Nadu Agricultural University, Coimbatore. 2013, 112.
10. Dillard HR, Hunter JE. Association of common ragweed with Sclerotinia rot of cabbage in New York state. Plant Dis. 1986; 70:26-28.
11. Farr DF, Bills GF, Chamuris GP, Rossman AY. Fungi on Plants and Plant Products in the United States. In: The American Phytopathological Society. 1989; 1252.
12. Fernando WGD, Nakkeeran S, Zhang Y, Savchuk S. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. Crop Protection. 2007; 26(2):100-107.
13. Gomez KA, Gomez AA. Statistical Procedure for Agricultural Research. John Wiley and Sons, New York, 1984.
14. Huang HC, Hoes JA. Importance of plant spacing and sclerotial position to development of *Sclerotinia* wilt of sunflower. Plant Dis. 1980; 64:81-84.
15. Huang HC, Bremery E, Hynes RK, Erickson RS. Foliar application of fungal biocontrol agents for the control of white mold of dry bean caused by Sclerotinia sclerotiorum. Biol. Control. 2000; 18:270-276.
16. Kamesh KK, Sankaralingam A. Morphological variations of sclerotia of Sclerotinia sclerotiorum produced on different substrates. Trends in Biosci. 2016; 9(5):364-366
17. Kamesh KK, Sankaralingam A. Efficacy of biocontrol agents in the management of head rot of cabbage (*Brassica oleracea var. capitata*) caused by Sclerotinia sclerotiorum. Afr. J. Microbiol. Res. 2016; 10(40):1711-1714.
18. Korf RP, Dumont KP. *Whetzelinia*, a new generic name for *Sclerotinia sclerotiorum* and *S. tuberosa*. *Mycologia.* 1972; 64:248-251
19. Mclean DM. Occurrence of *Sclerotinia* stalk rot in cabbage seed plants in Skagit County, Washington in 1948. Plant Dis. Rep. 1949; 33:222-223.
20. McLean DM. Role of dead flower parts in infection of certain crucifers by *Sclerotinia sclerotiorum* (Lib.) D By. Plant Dis. Rep. 1958; 42:663-666.
21. Mohiddin FA, Khan MR. Tolerance of fungal and bacterial biocontrol agents to six pesticides commonly used in the control of soil borne plant pathogens. Afr. J. Microbiol. Res. 1958; 8(43):5331-5334.
22. Purdy LH. *Sclerotinia sclerotiorum*: History, diseases and symptomatology, host range, geographic distribution and impact. Phytopathol. 1979; 69:875-880.
23. Rajesh P. Role of antimicrobial peptide genes of PGPR and fungicides for the management of stem rot and wilt complex of carnation under protected cultivation, *M.Sc. (Agri.) thesis*, Tamil Nadu Agricultural University, Coimbatore. 2013, 100.
24. Sendhilvel V, Marimuthu T, Raguchander T. Compatibility of azoxystrobin 25 SC with biocontrol agents. *Pestology.* 2004; 28:61-64.
25. Stelfox D, Williams JR, Soehngen U, Topping RC. Transport of *Sclerotinia sclerotiorum* ascospores by rapeseed pollen in Alberta. Plant Dis. Rep. 1978; 62:576-579.

26. Valarmathi P, Sushil KP, Priya V, Rabindran R, Gopal C. Compatibility of copper hydroxide (Kocide 3000) with biocontrol agents. *I.O.S.R.-J.A.V.S*., 2013; 3:28-31.
27. Vidhya PR, Napolean P, Balamurugan A, Jayanthi R, Beulah T, Premkumar R. *In vitro* studies of biocontrol agents and fungicides tolerance against grey blight disease in tea. Asian Pac. J Trop. Biomed, 2012, S435-438.
28. Xiaojia H, Daniel PR, Lihua X, Jude EM, Changbing Y, Yinshiu L *et al*. *Bacillus megaterium* A6 supresses *Sclerotinia sclerotiorum* on oilseed rape in the field and promotes oilseed rape growth. Crop Protection. 2013; 52:151-158.