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Research Article

Efficacy of substrate based bioformulation of microbial antagonists in the management of bacterial disease of some solanaceous vegetables in Assam

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ABSTRACT: A study was undertaken to explore effective organic substrate-based bioformulation using virulent cells of antagonists *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride* during 2010-13. Three organic substrates, *viz.* vermicompost, farm yard manure and mustard oil cake (MOC) were compared for mass multiplication of the antagonists. All the substrate based antagonists showed effective results in suppression of bacterial wilt (*Ralstonia solanacearum* Yabuchi *et al*.) incidence in vegetable crops tomato, brinjal and chill. Quantitative aspect of population dynamics of the antagonists at different days of storage was made to evaluate the shelf-life of the biopesticide and found that the antagonists maintained a steady population count upto 180 days of storage at room temperature. The combination of vermicompost *P. fluorescens*, carboxy methyl cellulose (CMC) and mannitol showed best shelf-life as it maintained highest population recovery of *P. fluorescens* and *B. subtillis* at different days of storage. The combination of MOC, *T. viride*, CMC and mannitol showed best shelf-life in case of *T. viride* and maintained highest population recovery of the antagonist at different days of storage. Application of the substrate based bioformulations as combination of seed treatment, root application, soil application at transplanting and soil application at 30 days after transplanting showed minimum wilt incidence and maximum yield in tomato, brinjal and chilli. Maximum disease reduction (81.85%) was shown by bioformulation comprising *P. fluorescens* with vermicompost as substrate followed by *T. viride* with MOC as substrate (79.07%). Following the trend of reduction in disease incidence, yield was maximum in tomato (36.0 t/ha), when the crop was treated with vermicompost based *P. fluorescens* followed by treatment with MOC based *T. viride* (33.35 t/ha). Yield of brinjal (27.60 t/ha) and chilli (26.30 t/ha) was similarly maximum when bioformulation of vermicompost based *P. fluorescens* was applied.

KEY WORDS: Antagonists, bioformulation, chilli, tomato, wilt

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INTRODUCTION

Solanaceous vegetables constitute a major group of economically important vegetables in Assam of which tomato, brinjal and chilli are most extensively cultivated in the state. Tomato cultivation covers an area of 12,550 hectares with productivity of 16.5 tonnes/hectare. Similarly, brinjal and chillies are grown in about 26,000 and 13,400 hectares with productivity of 15.2 tonnes/ha and 13.5 tonnes/ha, respectively. However, *Ralstonia solanacearum* incited wilt disease is one of the major yield limiting factors of solanaceous vegetables in Assam and causes severe losses ranging from 92 to 100 per cent, when environmental conditions become favourable for pathogen manifestations (Bora and Bora, 2008; Bora and Bora, 2009). Means to control *R. solanacearum* incited bacterial wilt disease are limited. Various tactics like crop rotation is not a viable as the bacterium is soil inhabitant and persist indefinitely in infested fields (Chellemi *et al.,* 1994), use of antibiotics frequently leads to development of resistance races of the pathogen (Sigee, 1993), use of resistant cultivar was most logical solution but break down of resistance is quite common due to intensive cultivation. To combat the limitation of these management practices, presently plant disease management has been directed towards the environmentally safe and economically feasible bio-intensive strategy as the alternative means for plant disease management. In this context, exploitation of saprophytic antagonists as biological control agent has been considered as suitable tactic, as it is environmentally safe and economically feasible. Strains of fluorescent pseudomonads, *Trichoderma* spp. and *Bacillus* spp. are known

antagonists against soil borne plant pathogens and attempts have been made throughout the world to explore the possibilities of using these saprophytic antagonists for crop disease management (Burr *et al.,* 1978; Papavizas, 1985; Bull, 1987; Anuratha and Gnanamanickam, 1990; Nautyal, 2000; Bora *et al.,* 2000; Bora and Deka, 2007; Bora, 2008; Bora and Bora, 2008).

In the light of these factors, present study was undertaken to explore the potential of an environment friendly strategy with bioformulation of *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* to combat disease of solanaceous vegetable crops (tomato, brinjal and chilli).

MATERIALS AND METHODS

Different experiments were conducted in the laboratory of the Department of Plant Pathology, Assam Agricultural University, Jorhat and farmers field of Sonitpur district. Diseased tomato, brinjal and chilli plants showing symptoms of bacterial wilt were used for the isolation of *R. solanacearum* using triphenyl tetrazolium chloride (TTC) medium. The inoculum concentration of the pathogen was always adjusted to a bacterial population of 1 x 10^8 colony forming units per milliliter (cfu/ml). The pure cultures of the antagonists were collected from the culture stock of the Department of Plant Pathology, AAU, Jorhat. King'B agar media and potato dextrose agar (PDA) media were used for the multiplication and preservation of *Pseudomonas fluorescens, B. subtilis* and *T. viride* respectively.

The organic substrates used for the mass multiplication of antagonists were vermicompost (VC), farm yard manure (FYM) and mustard oil cake (MOC). The substrates were air dried and passed through 350 mesh sieves to obtain fine powders. These were filled into polypropylene bags separately, sealed with non-absorbent cotton plugs and sterilized at 121°C for 30 minutes. Mass culture of fluorescent pseudomonad strain *P. fluorescens* was prepared by transferring aseptically its 24h old growth in KB agar into 1000 ml KB broth and incubated at 28°C for 24h. Similarly, mass cultures of *B. subtilis* and *T. viride* was prepared by transferring aseptically their 72h old growth in PDA to 1000 ml PD broth and incubated at 28°C for 120h. From these, 10 ml of the *P. fluorescens* and *B. subtilis* cells (10^7cfu/ml) and 10ml of *T. viride* cells (10^7 cfu/ml) respectively were added to the sterilized substrates contained in the polypropylene bags. A standard sticker, carboxy-methyl cellulose (CMC @ 1%) was added in order to impart greater adherence property and a standard osmoticant (Mannitol @ 3%) was added to impart higher moisture retaining property to the substrates. The inoculated substrates were then mixed properly and the mixtures of substrates + *P. fluorescens*; substrates + *B. subtilis*; substrates + *T. viride* along with CMC and Mannitol were incubated at 28°C for 72h. The bags were stored at room temperature after incubation.

For determination of the population of *P. fluorescens, B. subtilis* and *T. viride* in different substrate formulation after different days of storage as prepared above, experiment was designed following completely randomized design with 3 replications. The 10 different treatment combinations were : VC + *P. fluorescens*; VC + *B. subtilis*; VC + *T. viride*; FYM + *P. fluorescens*; FYM + *B. subtilis*; FYM + *T. viride*;MOC + *P. fluorescens*; MOC + *B. subtilis*; MOC + *T. viride*; and control. The viable population of *P. fluorescens*; *B. subtilis* and *T. viride* in different substrates was determined after 60, 120, 180, 240 and 300 days of inoculation following dilution plate technique to ascertain the best antagonist + substrate combination for highest shelf life of the formulation in storage at room temperature.

The field experiment was carried out with pot grown tomato, brinjal and chilli plants to evaluate the best method of application of the substrate based antagonists in management of bacterial wilt disease of these vegetable crops. All together 10 treatments were imposed, arranged in RBD and each replicated 4 times. The best substrate based bioformulation of *P. fluorescens, B. subtilis* and *T. viride* was applied in tomato, brinjal, and chilli as seed treatment, root treatment, soil treatment and their combinations at the time of transplanting and at 30 days after transplanting.

For seed treatment, seeds of tomato, brinjal and chillies respectively, were first cleaned individually and treated with the bioformulation @1 gm/gm of seed along with a sticker/adhesive like rice glue for easy adherence. The coated seeds were then spread over a clean paper and dried overnight. For root treatment of seedlings, the bioformulation was mixed with rice gruel to form fine slurry and the roots of the seedlings were dipped in it for about 30 minutes. For 1000 seedlings of each crop 1kg of bioformulation was required. The treated roots were dried for 1 hour under shade before transplanting. For soil treatment at the time of transplanting, 10 g of bioformulation was mixed with 100 g of vermicompost (@ 10 kg bioformulation mixed with 1qt vermicompost/ha) and was applied to the soil near the root zone of the crops. Similarly, the same treatment soil application of bioformulation was repeated at 30 days after transplanting. Observations were made on the wilt incidence (%) in each crop and yield (t/ha) for all the treatments in each crop.

RESULTS AND DISCUSSION

Population of antagonists in various substrate based formulations after different days of storage:

The mean population of all the three antagonists in formulations of three substrates significantly increased upto 180 days of storage after which it showed declining trend (Table 1). The highest population of *P. fluorescens* (112.43 x 10^7 cfu/g) was recovered from formulation where vermicompost was used as substrate. While the least population of *P. fluorescens* was recovered from the formulation where MOC was used as substrate. Similarly, the highest population (71.17 x 10^7 cfu/g) of *B. subtilis* was recovered when vermicompost was used as substrate, while least population was recovered when MOC was used as substrate. On the other hand, the highest population of *T. viride* (108.83 x 10^7 cfu/g) was recovered from formulation comprising of MOC as substrate after 180 days of storage. The lowest population of *T. viride* was recovered from the formulation comprising FYM as substrate. On an average, vermicompost among the substrates appeared to be the best nutrient source to support the antagonists for maximum multiplication and subsequently better disease reduction. Earlier, Suslow and Schroth (1982) reported that fluorescent pseudomonad *P. fluorescens* could survive 7 months to 1 year with a higher population level when it was incorporated into carrier materials *viz*., talc or peat along with CMC. The higher population of antagonists might be due to high nutrient content of vermicompost, which is a good source of humus, Vitamin-B, auxin and antibiotics. Moreover, it contains 2.5-3.5 per cent nitrogen, 1.5-2.0 per cent phosphorous and 2.0-3.5 per cent potassium (Kohli *et al.,* 1988). Vermicompost causes a shift of pH towards neutral, a reduction in electrical conductivity, and therefore fluorescent pseudomonad, *P. fluorescens,* which prefers neutral to alkaline pH tends to exhibit higher population shift in vermicompost (Alexander, 1997). CMC was used in the formulation as an adhesive, which might have also played a role of preservative for the long-term viability of the antagonist. Moreover, mannitol used as osmoticant, has the ability to protect the antagonist from desiccation and thereby increases their survivality (Vidhyasekaran and Muthamilan, 1995). Population dynamics of *B. subtilis* might have similarly supported by vermicompost, when it was used as substrate. However, *T. viride* showed higher population count when MOC was used as its substrate. MOC as substrate might have helped *T. viride* in better sporulation and production of colony forming units as it could release enzymes like β-1-3 glucanase and chitinase and these might have helped the fungi during utilization of cellulose and chitin present in different substrates (Hadar *et al.,* 1979). *Trichoderma* spp., multiply faster at higher concentration of CO_2, a condition favoured by MOC as substrate. Similarly, it might also have been favoured by humic acid present in the MOC (Ushasree *et al.,* 1989).

Efficacy of the substrate based formulations of *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* on reduction of bacterial wilt incidence in tomato, brinjal and chilli

All the treatment combinations, irrespective of antagonist and substrate used, were significantly effective in lowering wilt of tomato brinjal and chilli crops (Table 2). However, *P. fluorescens* along with vermicompost and *T. viride* along with MOC as substrate were significantly most effective in reducing disease incidence. Data also depicted that *B. subtilis* along with MOC as substrate was least effective in reducing disease incidence.

Maximum disease reduction (81.85%) was shown by bioformulation comprising *P. fluorescens* with vermicompost as substrate followed by *T. viride* with MOC as substrate (79.07%), while least disease reduction (54.33%) was shown by *B. subtilis* with MOC as substrate followed by *T. viride* with vermicompost as substrate (54.92%).

Better disease reduction in tomato caused by *R. solanacearum* was earlier recorded by using *P. fluorescens* as seed and seedling inoculation. Fluorescent pigments produced by the pseudomonads sequester Fe^{3+} and are considered siderophores, which inhibits large number of phytopathogenic bacteria and fungi in soil (Aspiras and Della Cruz, 1985). Similarly, Kloepper and Schroth (1981) reported that fluorescent pseudomonad could rapidly colonize and inhibit certain components of the root zone microflora and along with rich substrate the antagonist beneficially alter the composition of the rhizosphere leading to reduced plant disease incidence. However, *T. viride* with MOC as substrate was more effective in reduction of disease incidence in ginger. MOC might have helped *T. viride* in better sporulation, production of colony forming units and subsequent higher reduction of disease incidence in ginger. Danielson and Davey (1973) reported that *Trichoderma* spp., multiply faster at higher concentration of CO_2 and when substrates like MOC containing carbon are degraded, CO_2 is evolved. From above discussion, it is evident that substrates like vermicompost, FYM and MOC enhances the activity of antagonists, which complete with the soil borne plant pathogen for nutrient and space.

The addition of antagonists along with different substrates might have influenced of soil organic carbon. Earlier, Hoitink and Fahy (1986) tried to establish positive correlation between C : N ratio of residues of organic carbon and disease severity. Similarly, an increase in the available phosphorus content of soil has been reported to be effective in suppressing the disease incidence. The substrates particularly vermicompost increases available phosphorus content in the soil in contrast to the other treatment, which could provide maximum protection to the plants from the disease. The increased availability of phosphorus and potash in soil might have contributed towards the resistance of the plants to the diseases as have been recorded in many other pathogens (Sharif *et al.,* 2003).

Efficacy of substrate based bioformulation of *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma viride* on yield of tomato, brinjal and chilli

The yield of all the crops increased significantly when plants were treated with *P. fluorescens, B. subtilis* and *T. viride* based bioformulation, following different methods (Table 3). Following the trend of reduction in disease incidence, yields was maximum in tomato (36.0 t/ha), when the crop was treated with vermicompost based *P. fluorescens* applied as seed treatment + root treatment + soil application at transplanting + soil application at 30 days after transplanting (DAT). This was followed by treatment with MOC based *T. viride* (33.35 t/ha). Yield of brinjal (27.60 t/ha) and chilli (26.30 t/ha) was similarly maximum when bioformulation of vermicompost based *P. fluorescens* was applied. Least yield of brinjal (17.33 t/ha) and chilli (15.42 t/ha) was recorded in the treatment comprising bioformulation of MOC based *B. subtilis.*

Successful biological control agents like fluorescent pseudomonads, *B. subtilis* and *T. viride* have the ability to compete with other members of the soil microflora and also to produce antibiotics or induce a response in the host that favours growth of the plant beneficial microbes while inhibiting the growth of the pathogen like *R. solanacearum.* Simultaneous application of two or more compatible antagonists led to their higher population densities in the rhizosphere and the density dependent mechanism might have prevailed by rapid rhizosphere colonization, and thereby led to rhizosphere niche exclusion in terms of space and nutrients for the pathogen and ultimately reduction of wilt incidence and corresponding enhancement of crop yields. Such mechanisms have been demonstrated by Bull (1987), and explained that the wheat take-all disease control and enhancement of crop yield by biological means was directly related to the rapid root colonization by antagonist like *P. fluorescens.* Moreover, the efficiency of biocontrol agents with organic substrates is depended on method of introduction, rate of inoculum density of the

Table 1. Population of *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma viride* assayed from substrate based bioformulations after different days of storage

TREATMENTS	Population of antagonists (x 10^7 cfu/g) after different days of storage					
	60	120	180	240	300	Mean
Vermicompost (VC) + *P. fluorescens*	64.33	90.50	112.43	86.10	16.48	73.97
	(1.80)	(1.96)	(2.05)	(1.93)	(1.21)	(1.86)
Farm yard manure (FYM) + *P. fluorescens*	34.50	60.00	72.88	57.87	07.45	46.54
	(1.54)	(1.77)	(1.86)	(1.76)	(0.87)	(1.66)
Mustard oil cake (MOC) + *P. fluorescens*	16.50	26.50	27.89	22.17	05.30	19.67
	(1.22)	(1.42)	(1.44)	(1.35)	(0.72)	(1.29)
VC + *T. viride*	42.55	64.50	75.66	74.27	02.15	51.83
	(1.63)	(1.81)	(1.66)	(1.87)	(0.33)	(1.71)
FYM + *T. viride*	25.99	49.50	51.50	33.10	01.50	32.32
	(1.41)	(1.69)	(1.71)	(1.52)	(0.17)	(1.51)
MOC + *T. viride*	62.83	91.17	108.83	71.50	07.50	68.37
	(1.80)	(1.96)	(2.04)	(1.85)	(0.30)	(1.83)
VC + *B. subtilis*	43.50	59.50	71.17	39.06	04.50	43.55
	(1.64)	(1.77)	(1.85)	(1.59)	(0.87)	(1.64)
FYM + *B. subtilis*	21.27	52.40	48.17	35.50	01.50	31.77
	(1.33)	(1.72)	(1.68)	(1.55)	(0.17)	(1.50)
MOC + *B. subtilis*	15.50	16.50	34.80	09.30	00.50	15.32
	(1.19)	(1.22)	(1.54)	(0.96)	(0.30)	(1.18)
Mean	36.33	56.67	67.04	47.65	05.20	
	(1.56)	(1.75)	(1.82)	(1.68)	(0.72)	

S.Ed. ± = For treatment = 0.022, for days = 0.016, for treatment x days = 0.042; CD (*P*=0.05) = For treatment = 0.044, for days = 0.032, for treatment x days = 0.084; Figures in the parentheses are logarithm-transformed values.

Table 2. Effect of substrate based bioformulations of *Pseudomonas fluorescens, Trichoderma viride and Bacillus subtilis* on reduction of bacterial wilt incidence in tomato, brinjal and chilli

Treatments	Wilt incidence (%)*	Disease reduction over control (%)
Vermicompost (VC) + *P. fluorescens*	17.17 (22.19)[a]	81.85
Farm yard manure (FYM) + *P. fluorescens*	29.15 (30.55)[b]	69.19
Mustard oil cake (MOC) + *P. fluorescens*	37.75 (35.25)[c]	62.00
VC + *T. viride*	42.65 (36.56)[c]	54.92
FYM + *T. viride*	24.40 (29.24)[b]	74.21
MOC + *T. viride*	19.80 (25.37)[a]	79.07
VC + *B. subtilis*	25.20 (30.71)[b]	73.36
FYM + *B. subtilis*	37.20 (34.75)[c]	60.68
MOC + *B. subtilis*	43.20 (37.90)[c]	54.33
Control (Untreated)	94.60 (81.55)[d]	-
SEd (±)	(1.62)	
CD(*P*=0.05)	(3.24)	

* Figures in the parentheses are angular transformed value

Table 3. Effect of substrate based bioformulations of *Pseudomonas fluorescens, Trichoderma viride and Bacillus subtilis* on enhancement of yield in tomato, brinjal and chilli

Treatments	Tomato yield (t/ha)	Brinjal yield (t/ha)	Chilli yield (t/ha)
Vermicompost (VC) + *P. fluorescens*	36.10	27.60	26.30
Farm yard manure (FYM) + *P. fluorescens*	32.90	21.20	21.20
Mustard oil cake (MOC) + *P. fluorescens*	27.30	19.40	17.40
VC + *T. viride*	30.40	24.60	23.50
FYM + *T. viride*	24.00	21.40	20.60
MOC + *T. viride*	33.35	27.40	31.80
VC + *B. subtilis*	23.93	24.98	21.77
FYM + *B. subtilis*	21.45	19.65	16.45
MOC + *B. subtilis*	19.66	17.33	15.42
Control (Untreated)	01.65	01.98	01.22
SEd (±)	01.44	01.15	01.65
CD(*P*=0.05)	02.88	02.30	03.30

bioagents applied against the pathogens (Papavizas, 1985). Parakhia and Vaishnav (1986) observed that seed treatment with wheat husk-bran culture of *T. harzianum* followed by soil drenching could successfully control *R. bataticola* infection in chick pea and increase crop yield. Similarly, Vidya (1995) used combination of talc-based formulation of *T. harzianum* + vermiculite-wheat bran formulation for soil application and effectively managed *M. phaseolina* root rot disease of mung bean resulting higher crop yield.

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