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ETIOLOGY AND MANAGEMENT OF TIP-OVER DISEASE OF BANANA BY USING BIOLOGICAL AGENTS

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ABSTRACT
Tip-over disease of banana is becoming a serious problem in all the banana growing areas of Kamataka and Andhra Pradesh states of India particularly two to six months old gardens planted with tissue culture plantlets belonging to cv. G-9 and Robusta. The disease incidence ranged from 30-35 per cent in the districts of Bangalore and Kolar of Karnataka state. The disease caused rotting of rhizome and pseudo stem, following marginal necrosis or scorching of leaves ultimately leading to toppling of affected plants. The nine isolates of the bacterium were identified as *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi* on the basis of morphological, biochemical and physiological and pathogenicity tests. The Bijapur (I_7) and Andhra Pradesh (I_9) isolates varied with respect to some biochemical and physiological characteristics. The antagonistic, bacteria viz., *Bacillus subtilis* and *Pseudomonas fluorescens* gave good control of the disease followed by VAM fungi (*Glomus fasciculatum*). The modified organic formulation Panchagavya (MPG-3) was also found to be effective in controlling the disease as compared to water extracts from *citronella* and *Clocimum*.

KEY WORDS: 'Tip-over' disease, *Erwinia carotovora* subsp. c*arotovora*, *Oscimum, Closcimum*, Modified Panchagavya.

INTRODUCTION

Banana is one of the oldest cultivated tropical fruits in India next to mango in both area and cultivation. Kamataka occupies second place in area and third place in production in India. The plant is subjected to attack by many serious debilitating diseases caused by various pathogens. Among the bacterial diseases, moko disease is a serious problem in several parts of the world, however tip-over or bacterial rhizome rot disease, which was considered to be minor earlier, has assumed serious proportions in recent years, in India in general and Kamataka in particular. With the advent of tissue culture technique for the mass production of banana plant in view of increasing demand due to the rapid expansion of banana cultivation, the disease is spreading fast causing high plant mortality, consequent losses to the planters. A soft rot disease of banana referred to tip-over caused by *Erwinia carotovora* was recorded in Honduras in 1949 (Wardlaw, 1950 and Stover, 1959). Hildreth (1962), recorded losses as high as 80-90 % and upto 93% in Gautemala. The disease was recorded in India by Edward *et al*., (1973) and Khan and Nagaraj (1998) recorded the incidence of Tip-over disease of banana upto 70 per cent in Karnataka. There are conflicting reports pertaining to the exact identity of the causal organism. Several workers in the past have reported it to be *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi* from across the world (Dickey and Victoria, 1980; Choi *et al*., 1988; Stover, 1959). From India it was reported to be *Erwinia carotovora* subsp. *carotovora* (Edward et al. 1973; Lakshmanan and Mohan, 1980 and Khan and Nagaraj, 1998). While, Chattopadhyay and Mukherjee (1986) attributed it to be *Erwinia chrysantherni*. Though chemical control has been found to be effective, but needless to say that they are hazardous to environment and human health, therefore alternate control measures need to be developed. Hence the investigations were undertaken to study the symptoms, identity of the causal organism and develop suitable bio-control measures, which has a great potential as a management strategy for a fruit crop like banana.

MATERIAL AND METHODS

Symptomatology: The disease occurrence and symptoms of the disease was studied by undertaking the survey of the various banana gardens in the major banana growing areas of Karnataka state.

Isolation of causal organism and Identification

Banana plants showing symptoms of Tip-over such as rhizome rot accompanied by massive soft rot of the rhizome at the central and peripheral region were collected from different agro climatic areas of Karnataka and Andhra Pradesh. The affected rhizomes were washed in running tap water and the rotted over portions was chaffed off exposing the dark brown-black discoloured area. The infected tissues cut aseptically into small pieces were surface sterilized in one percent sodium hypochlorite for two minutes and washed in sterile distilled water. The bacterium isolated by ooze method followed by spread plate on nutrient agar. The plates were incubated at 28° C for 48 hours. Well separated shiny, creamy white, mucoid, regularly shaped colonies were picked up and purified and the isolates were designated as I_1 to I_9. The identification of the bacterial isolates on the basis of morphological, cultural, and bio-chemical and pathogenicity

characteristics as described by Bradbury (1970), Dickey and Victoria (1980) and Schaad (1992). The pathogenicity test was carried out by inoculating 48 hours old bacterial culture to 30 days old banana seedling cv. G-9 at the collar region of the pseudostem with a thick suspension of 7 x $l0^7$ cfu/ml under *in-vivo* conditions.

Biological control of tip-over disease

A filed trial was conducted during the Kharif season on Robusta banana to test the efficiency of various bio-control agents, viz., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Glomus fasciculatum* and the plant extracts viz., *Citronella*, *Closcimum* and an organic formulation the modified Panchagavya [MPG-3] at two places in Bangalore districts, . The antagonistic microorganism's viz., *Pseudomonas fluorescens* and *Bacillus subtilis* were mass multiplied in nutrient broth and the cell suspension was adjusted to a concentration of 5 x 10^7 cfu/ml turbidometrically and applied around the pseudostem of banana plants 20 days after planting. The VAM fungi (*Glomus fasciculatum*) obtained from the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore was mixed in the soil around the root zone of 20 days old plants at a rate of 5g/plant containing 500 to 600 chlamydospores/g. While the undiluted plant extracts of the plant species was drenched in the soil around the pseudostem at 15ml/plant and the trial was laid out as per the RCBD design. The treatments were imposed thrice at weekly intervals except for VAM fungi (*Glomus fasciculatum*). Observations were recorded on the percent disease incidence and various yield parameters at weekly intervals.

RESULTS & DISCUSSION

Below ground symptoms

Survey conducted in two major banana growing districts i.e., Bangalore and Kolar revealed that the incidence of disease in the range of 30-35 per cent in two popular varieties of banana G-9 and Robusta in 2-6 month old crop. The chief symptoms of the disease are rotting of rhizomes accompanied by brown discolouration starting from the periphery of the rhizome and extending laterally towards the central core, immediately the rotting extends to cover large portion of the rhizome. The affected tissue become soft, mushy and turn brown to black and at this stage tunneling in the rotted area was observed, which could be confused for the weevil infestation. As the disease advances the rotting engulfs the central portion of the rhizome involving the collar region and occasionally moving up to the pseudostem. The young affected plants topple over due to wind or just mild knock with hand.

Above ground symptoms

The affected plants appear weak, dwarf with pale yellow luster less leaves. The marginal necrosis or scorching of the lower leaves was also observed. Sometimes the young newly emerging leaves fail to open and appear brown and necrotic leading to arresting the plant growth. The symptoms on the leaves could be correlated with extent and region of the rhizome damaged. The symptoms on the leaves could be correlated with extent and region of the rhizome damaged. Similar symptoms were reported by Stover, (1959), Chattopadhyay and Mukherjee (1986), Periera and Nunes (1988) and Khan and Nagaraj (1998).

Isolation of the causal organism

Repeated isolations made from the affected rhizomes obtained from different parts of Karnataka and Andhra Pradesh consistently yielded creamish yellow, mucoid, shiny, convex and round to irregular colonies on nutrient agar. The isolates were designated as I_1 to I_9. The Bijapur isolate (1_7) produced slightly dark yellow colonies and were moderately mucoid, while the isolate 1_9 from Kovvur, Andhra Pradesh produced cream white, moderately shiny, slightly flat, round to irregular colonies. The results of the morphological, physiological and biochemical characters of the nine isolates are presented in Table 1. All the isolates behaved similarly with respect to the various characteristics studied, except for isolates 1_7 and I_9 with respect to lactose, trehalose, maltose, gelatin liquefaction, sensitivity to erythromycin, acetoin production and production of acid in media containing various sugars (Table-1). All the nine isolates produce the typical disease symptoms after 25 days of inoculation. However, the isolates I_7 took slightly longer time to produce the symptoms. All the isolates produce pits in the CVP agar media indicating that the pectolytic activity, which is a typical character of *Erwinias* (Naumann and Scmidt 1979; Havesi *et al*., 1981). Thus on the basis of various bio-chemical and physiological arid pathogenicity characteristics exhibited by the isolates I_1, I_2, I_3, I_4, I_5, I_6 and I_8 the isolates were identified as belonging to *Erwinia carotovora* subsp. *Carotovora.* The isolate I_9 from Kovvur in Andhra Pradesh expressed characteristics similar to that of *Erwinia chrysanthemi*, hence designated as *Erwinia chrysanthemi* (Schaad 1992, Dickey and Victoria 1980), while I_7, which showed wider variation possessing characteristics in between the above two species of *Erwinia*. Similar observations were also reported by Hassanzadeh (1990), from Iran who observed characteristics of 10 isolates of banana intermediate between *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi*.

Biological control

The data on the field trial conducted using bio-control agents revealed that *Bacillus subtilis* totally suppressed the disease (100 %) followed by *Pseudomonas fluorescens* and VAM fungi (*Glomus fasciculatum*) in which 87.5 per cent control was observed over control. The organic amendment i.e., modified, Panchagavya controlled the disease incidence by 75 per cent while the two plant extracts gave 62.5 per cent control each. Higher increase in yield was recorded in *Bacillus subtilis* treated plants (85. 7%) followed by VAM fungi and modified Panchagavya (Table 2 & 3).

TABLE 1: Comparative morphological, physiological and biochemical characteristics of the nine isolates of *Erwinia* causing tip – over disease of banana

Sl. No.	Isolates I_1 to I_6 and I_8	I_7	I_9
Growth on NA	Colonies were light Yellow, mucoid, convex, glistening, round to irregular.	Colonies were dark yellow, slightly mucoid, Moderately convex round to irregular.	Colonies were creamy white, flat, non-mucoid, round to irregular
II Morphology			
1. Shape	Small rods	Small rods	Small rods
2. Occurrence	In singles	In singles	In singles
III Staining			
1. Gram staining	Negative	Negative	Negative
2. Spore staining	Non spore forming	Non spore forming	Non spore forming
3. Capsule	Non capsulated	Non capsulated	Non capsulated
4. Flagella staining	Peritrichous	Peritrichous	Peritrichous
IV Biochemical Characteristics			
1. Pectate degradation	+	+	+
2. Potato soft rot	+	V	+
3. Gelatin liquefaction	-	+	-
4. Acetoin production	+	-	+
5. Sensitivity to erythromycin	-	+	+
6. Gas from glucose	+	-	+
7. Indole production	-	-	-
8. Reducing substances from sucrose	-	+	+
9. Growth at 36-37 ^{o}C	+	+	+
10. Acid from lactose	++ (2 days)	++ (2 days)	+weak (4 days)
11. Trehalose	++	+weak	++
12. Maltose	++	+weak	++
13. Cellobiose	++	+weak	++
14. Catalase reaction	+	+	+

*V-In one test the Isolate cause soft rot, but in subsequent experiments it did not.

TABLE: 2 Effect of bio-control agents on the tip-over disease of banana caused by *Erwinia carotovora* subsp. *carotovora* under field conditions in Doddabelavangala, Bangalore rural district

Sl. No	Treatments	No. of plants per treatment	Number of plants infected (percent disease incidence)						
			Before application	After I drench	After II drench	30 DAIII drench	60 DA III drench	90DA III drench	% Redn. Over control
1	*Pseudomonas fluorescence*	9	4(44.44)	4(44.44)	3(33.34)	2(22.23)	1(11.12)	1(11.12)	87.49
2	*Bacillus subtills*	9	3(33.34)	3(33.34)	2(22.23)	1(11.12)	0(00.00)	0(00.00)	100
3	*Glomus fasciulatum*	9	3(33.34)	3(33.34)	2(22.23)	2(22.23)	2(22.23)	1(11.12)	87.49
4	Citronella	9	4(44.44)	4(44.44)	4(44.44)	3(33.34)	3(33.34)	3(33.34)	62.5
5	Clocimum	9	5(55.55)	5(55.55)	4(44.44)	4(44.44)	4(44.44)	3(33.34)	62.5
6	Panchagavya(MPG-3)	9	4(44.44)	4(44.44)	3(33.33)	3(33.34)	2(22.23)	2(22.23)	75
7	Control	9	6(66.66)	7(77.77)	7(77.77)	8(88.88)	8(88.88)	8(88.88)	0
Grand Mean			46.06	47.61	39.68	36.5	31.74	28.57	
	Sem ±		8.61	9.88	8.44	10.91	9.27	8.26	
	CD at (5%)		27.13	31.13	26.59	74.39	29.23	26.03	

TABLE-3: Biological control of tip-over disease of banana and its influence on yield parameters in field trial conducted in Jakkur in Bangalore rural District.

Sl. No.	Treatment	Total no. of plants	No. of hands per bunch	No. of fingers per hand	Total yield (Kgs)	% Increase over control
1	*Pseudomonas fluorescens*	9	7.33	19.33	285	69.64
2	*Bacillus subtilis*	9	8.66	23	312	85.71
3	*Glomus fasciculatum*	9	8	21.66	300	78.57
4	Citronella	9	6.33	16	231	37.50
5	Clocimum	9	6	16	222	32.14
6	Panchagavya(MPG-3)	9	7.33	20.67	285	69.64
7	Control	9	5	12.33	168	0
Grand mean			6.88	18.05		
Sem ±			0.5	1.19		
CD at (5%)			1.58	3.76		

CONCLUSION

The nine isolates of the bacterium were identified as *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi* on the basis of morphological, biochemical and physiological and pathogenicity tests. The Bijapur (I_7) and Andhra Pradesh (I_9) isolates varied with respect to some biochemical and physiological characteristics. The antagonistic, bacteria viz., *Bacillus subtilis* and *Pseudomonas fluorescens* gave good control of the disease followed by VAM fungi (*Glomus fasciculatum*). The modified organic formulation Panchagavya (MPG-3) was also found to be effective in controlling the disease as compared to water extracts from *Citronolla* and *Clocimum*.

RECOMMENDATIONS

The bio control agents *Viz*., *Pseudomonas fluorescence, Bacillus subtillis* and *Glomus fasciulatum* can be recommended for the control of Tip-over disease of banana caused by *Ervinia carotovora* subsp. *Carotovora* along with modified Panchagavya (MPG-3). Further a study can be taken by adding all these combination and if it gives a good result, we can recommend in combination so that it controls the pathogen and also improves soil health.

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