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Elevated temperature and heat wave stress enhances the tip-over disease in banana in tropical plantation

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Abstract
The study was undertaken to ascertain the role of temperature and heat wave for causing of soft rot (or) head rot disease or tip over disease in Banana caused by *Erwinia ca rotovora* sub sp. *carotovora*. Banana crop is cultivated in Vellore District of Tamil Nadu nearly 3200 ha. The major problem in banana cultivation is tip-over disease during the month of March to May for past few years. The disease incidence is ranged from 34.6 to 67.9 per cent. The occurrence of disease was higher in tissue culture banana plant *viz.*, Grand Naine (G 9), followed by Karpooravalli and Poovan varieties. The highest temperatures ranged from 39.6 to 42°C were recorded during the month of April 2012, 2013 and 2014 and the maximum disease incidence was recorded was 67.9 per cent. The banana pseudostem contains about 90 per cent of water (stem juice). The stem juice is boiled during the month of April due to increasing higher temperature of 42°C. These temperatures caused the scorching of cigar leaf (recently emerging leaf still rolled as a cylinder) and predispose the tender tissue for rotting by saprophytic life phase of *E. carotovora sub sp. carotovora*. The leaf was tightly coiled and fragile in nature. The abiotic stress was supervise by sowing of *Sesbania grandiflora* as border crop and *Sesbania aculeate* as intercrop in banana field revealed that the tip-over disease incidence was reduced to 6.73 from 67.9 per cent recorded in the control plot.

Keywords: tip-over, temperature, green manure, varieties and *Erwinia carotovora*

Introduction
In India, banana rhizomes rot or soft rot caused by *Erwinia cartovora* sub sp. *carotovara* was a major problem in farmer's fields where tissue culture banana (Grand naine) was grown. The severity of the disease ranged from 30-80 per cent (Anon., 2005). Crop yield loss in the field ranged from 10 to 70 per cent. Rhizome rot caused by *Erwinia* spp genus known for its ability to cause soft rots with yield loss ranged from 30 to 45 per cent. These bacteria infect banana plants through leaves and pseudstems, causing the following symptoms like wilting or death of leaves before fruit has ripened, vascular discoloration and internal rot of the pseudostem (usually accompanied by a characteristically foul odor) Scot C. Nelson *et al.*, (2006).

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 banana farmers. 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) [22, 19]. Hildreth (1962) [11] recorded losses high as 80-90 per cent and upto 93 per cent in Gautemala. The disease was recorded in India by Edward *et al.*, (1973) [8]; Khan; Nagaraj (1998) recorded the incidence of tip over disease of banana upto 70 per cent in Karnataka

The bacteria are Gram-negative, non sporing, facultative anaerobes characterized by the production of large quantities of extracellular pectic enzymes. They rely mainly on the production of these enzymes together with a wide range of other plant cell wall-degrading enzymes to cause disease (Collmer & Keen, 1986) [5]. Additional pathogenicity-associated characters have been identified, which could also be involved in the establishment of the bacteria in plant tissues and in a free-living or saprophytic life phase. They are the main cause of tuber decay in store and blackleg or stem rot in banana field (Pérombelon, 1992) [16]. All *Erwinia* strains caused an economically important disease for which there is no chemical treatment (Toth *et al*., 2003) [21]. A number of factors have been reported to be responsible for tip over development *viz.*, host injury due to bruises, insects' damage, high humidity and high temperature. Since temperature is an important factor for destructive nature of the tip over disease it is imperative to study the disease development behavior and resulting economic losses incurred. The tip over disease is an emerging disease with new etiology due to climate variation, the studies on the correlation with heat wave and disease incidence was very limited.

As the disease etiology was new, a detailed investigation were undertaken to study the role of temperature and heat wave for the banana tip over disease development and management strategy through cultural methods.

Material and methods

Symptomatology

The occurrence of the tip over disease was noticed during the summer month of March to June in tropical area. The symptom was studied by undertaking the survey in different blocks of Vellore district in Tamil Nadu, India. The block level temperature and radiation is also recorded during the experiments.

Isolation of causal organism and Identification

The infected 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 blocks of Vellore district. The scorched tissues of cigar leaves were collected 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. The isolates were identified by based on the morphological, cultural and bio-chemical and pathogenicity characteristics as described by Bradbury (1970), Dickey and Victoria (1980) [6] and Schaad (1992).

Pathogenicitytest

The purified culture of *E. carotovaora sub.Sp carotovaora* were taken and suspended in the sterile distilled water with a load of 5 X 10 ^{6}cfu/ml. The cigar leaf and pseudostem was collected from the young two month old banana plants of grand naine, karpooravalli and poovan varieties. The leaves were cut in to circle shape with a diameter of four cm and placed in the sterile petriplate under moist condition using cotton. The pure culture was inoculated by pin prick method and incubated at 37ºC in incubator. Similarly the surface sterilised young pseudostem was inoculated and placed moistened petri plate with cotton.

Effect of green manure crop for the reduction of disease intensity

The experiment was laid out in factorial randomized complete block design (FRBD) and replicated three times. The treatments were allotted randomly to each replication. Details of the treatment along with symbols summarized as T_1- Agathi plant (*Sesbania grandiflora*) border plan T_2 - T_1 + *Sesbania* inter crop T_3 - T_1 + Indigo plant as inter crops T_4 - T_1 + Sun hemp T_5 -*Sesbania* inter crop alone T_6 - Indigo plant as inter crop alone T_7 - Sunhemp as inter crop alone T_8 – Control. The trial was initiated during the month of February, 2012 with grand naine variety. The factors used were sowing of green manures legumes in banana field and sowing of agathi plant (*Sesbania grandiflora*) as a border crop. Three legumes {Sesbans, *Sesbania aculeata* (L.); Indigo, *Tephrosia purpurea* (kolinji) Sunhemp, *Crotalaria juncea* (L.)} were used as inter crops and followed in situ incorporation green manure crops. There was a combination of eight treatments consisting of a fallow border and non border with agathi trees. The legumes were sown on 15 days after planting of banana variety grand nine. The border crop *viz.*, Agathi (*Sesbania grandiflora*) was sown in around the field with a distance 5 feet on the day of banana planting. The green manure crops were incorporated after reaching 2.5 feet height (45 days after sowing) during the onset of South West monsoon of June month.

Result and discussion

Symptomatology and temperature of pseudostem

The occurrence of the symptoms of tip over disease and pseudo stem temperature was recorded for three years periodically and presented in the Table 1. The manifested symptoms are scorching of cigar leaf inside pseudostem followed by rotting. The rotting was extended to pseudostem and then rhizome before emergence of the leaf. The partially dried leaves were shown tip and leaf blades are dried and brittle in nature. The rotten pseudostem and rhizome were shown mucos and oozing of Greyish-white or creamy white colour digested stem fluid. The rotten pseudostem was shown foul smell with maggots and other saprophytes. The higher temperature the favours the progression of the rotting and the affected plants were toppled down due to the complete rotting at collar region even at mild wind speed. The reactions of the symptoms were varied from variety and the severity was higher in grand naine followed by karpooravalli and poovan varieties. The similar types of symptoms were reported by Dipak *et al.*, 2013 [7], Nagaraj *et al.*, 2012 [14], Chattopadhyay and Mukherjee (1986) [3], Periera and Nunes (1988) [15].

Table 1: Occurrence of tip over disease symptoms in banana crop at different blocks of Vellore District

S. No	Name of the Block	Symptoms in banana varieties and pseudo stem juice temperature																		
		Scorching of cigar leaf			Rotting of cigar leaf			Partial drying / scorching of leaf			Complete drying of leaf			Pseudostem infection			Temperature of pseudostem juice		
		G 9	KV	Poovan	G 9	KV	Poovan	G 9	KV	Poovan	G 9	KV	Poovan	G 9	KV	Poovan	G 9	KV	Poovan
1.	K. V. Kuppam	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42.6	39.4	40.6
2.	Gudiyatttam	+	+	-	+	+	-	+	+	+	+	-	-	+	+	+	43.9	41.2	42.1
3.	Anaicut	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	38.9	37.6	35.4
4.	Kaniyambadi	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	37.6	33.6	33.5
5.	Katpadi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	36.5	37.4	36.7
6.	Vellore	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	37.8	38.6	37.5
7.	Madhanur	+	-	+	+	-	-	+	-	+	+	-	-	+	-	-	35.5	32.4	32.4
8.	Alangayam	+	+	-	+	-	-	+	-	+	+	-	-	+	+	-	37.9	34.6	35.7
9.	Natrampalli	+	+	-	+	-	-	+	-	+	+	-	-	+	+	-	41.5	36.5	37.8
10	Kandili	+	-	-	+	+	-	+	+	+	+	+	-	+	-	-	43.5	38.6	39.7
11	Thirupathur	+	+	-	+	+	-	+	+	+	+	+	+	+	-	+	42.7	34.5	40.1

G 9: Grand Naine KV: Karpooravalli

\+ Occurrence of the symptoms

\- Non occurrence of the symptoms

Isolation of the causal organism and pathogenicity

Isolations were made from in the infected site of all the three varieties such as grand naine, karpooravalli and poovn and isolates were designated as I_1, I_2 and I_3 respectively. The morphological characters were examined and biochemical tests were conducted (Table 2). The bacterium associated with the symptoms were identified as *E. carotovora* sub sp. *Carotovora* on the basis of morphological and bio chemical characters. The results pertaining to morphological and biochemical tests correlated with Chattopadhayay and Mukherjee, 1986; Tomlinson and Cox, 1987; Janse and Ruissen, 1988; Dipak *et.at*, 2013 and Nagaraj *et al.,* 2012) [3, 20, 15, 7, 14]. The present investigation of causal agent was supported by all the three isolates were inoculated and the produced peculiar symptoms as explained the symptomatology after 9 days after inoculation. The I3 isolated obtained from Poovan variety also took 12 days and correspondingly shows resistance in the field level. Similarly the tip over disease incidence was recorded by Vijayalaxmi., *et al.,* 2014 [17] at Karnataka.

Table 2: Morphological and biochemical tests for the confirmation of *E. carotovora var. carotovora strains*

Sl. No	Morphological tests						
	Morphological character	**Grand Naine**		**Karpooravalli**		**Poovan**	
1	Colonies	Greyish-white smooth, round		Dark yellow smooth, round to irregular		Dark yellow smooth, round	
2	Growth on agar medium	Facultative anaerobe		Facultative anaerobe		Facultative anaerobe	
3	Form	Occurring singly, unpairs, occasionally short chains		Occurring singly, unpairs, occasionally short chains		Occurring singly, unpairs, occasionally short chains	
4	Elevation	Slightly raised visible to the unaided eye		Slightly convex and glistening			
Bio chemical							
		Positive	**Negative**	**Positive**	**Negative**	**Positive**	**Negative**
5	Gram staining	No	Yes	No	Yes	No	Yes
6	Starch hydrolysis	No	Yes	No	Yes	No	Yes
7	Gelatin hydrolysis	Yes	No	Yes	No	Yes	No
8	Hydrogen sulphide production	No	Yes	No	Yes	No	Yes
9	Indole production	Yes	No	Yes	No	Yes	No
10	Methyl red test	Yes	No	Yes	No	Yes	No
11	Nitrate reduction test	Yes	No	Yes	No	Yes	No
12	Caesin hydrolysis	No	Yes	No	Yes	No	Yes
13	Sensitivity to erythromycin	Yes	No	Yes	No	Yes	No
14	Sensitivity to penicillin	No	Yes	No	Yes	No	Yes
15	Growth at 37° C	Yes	No	Yes	No	Yes	No

Effect of the green manure crop for the reduction of disease incidence

The field trial aimed to reduce the temperature and heat wave was conducted and the results were presented in Table 3. It is clearly indicated that the border crop with agathi tree (*Sesbania grandiflora*) and inter cropping with *sesbania* was found to be effective to reduce the disease incidence (6.9 %) and micro level temperature was 29ºC against the control (42 ºC). The present study was supported by Zang, 2013 stated that intercropping and rotating banana with Chinese chive can control panama disease and increase crop land biodiversity. The intercropping in banana increased the land use efficiency and reduced the pest and disease in banana (Wortmann, 1992) [2]. Smadja *et al.* (2004) [18] investigated the effects of various temperatures (8°C to 28°C) on maximum specific growth rate of *E. carotovora* sub sp. *Carotovora*. They noticed no growth for *E. carotovora* sub sp. *Carotovora* and low growth for *E. carotovora* sub sp. *Carotovora* at 8°C and observed an optimum growth for *E. carotovora* sub sp. *Carotovora* above 28°C. Laurent *et al.* (2001) [13] observed that the growth of *E. carotovora* sub sp. *Carotovora* was optimal at 28°C.

In a maize- bean intercropping system *Fininsa* (2001) reported that significant difference in microclimate between the sole crop and intercropping systems. Generally, temperature and wind velocity were lower in the intercrop than in the sole crop. Whereas relative humidity was higher. *Fininsa* (2001) monitored microclimatic temperature in sole and three bean- maize intercropping systems (e.g. row intercropping, mixed intercropping within row and broadcast intercropping). He found that the temperature was consistently more stable in the intercropping systems, *i.e.* cooler during the day (3°C) and warmer during the evening and night (0.9°C) also relative humidity increased during the day (8%) and decreased during the evening and night (2.6%).

The reduction of the temperature was ranged from 12 ºC in the combination of border and intercrop with *Sesbania*. It is clearly indicated that the border crop with intercrop reduced the heat wave and temperature in inside the field. The higher temperature in the control plot 42ºC was predisposed the host for maximum disease progression of 67.9 per cent and it was positively correlated with findings of the bacterial growth at 36-37ºC were reported by Goton (1979) Dicky and Victoria (1980), Bradbury (1986) [1], Tomlinson and Cox (1987) [20] and Chellemi *et al.* (1998) [4].

Table 3: Effect of cropping pattern against the tip over disease in banana caused by *Erwinia carotovora sub spcarotovora*

Treatments	Percentage of disease incidence			Temperature (ºC)		Mortality of the plants (%)
	30 DAP	60 DAP	90 DAP	Inside the field	Outside the field	
T_1 - Agathi plant (*Sesbania grandiflora*) border plan	13.74	23.52	31.23	33	39	27.33
T_2 - T_1 + Sesbania inter crop	2.43	4.43	6.73	29	41	5.62
T_3 - T_1 + Indigo plant as inter crops	11.42	15.66	27.82	32	39	23.4
T_4 - T_1 + Sun hemp	4.31	7.89	9.98	28	37	6.93
T_5 -Sesbania inter crop alone	9.77	11.43	13.86	32	39	11.12
T_6 - Indigo plant as inter crop alone	14.56	24.63	38.93	37	40	32.32
T_7 - Sunhemp as inter crop alone	8.56	12.3	14.56	31	42	12.86
T_8 - Control	17.9	33.8	67.9	39	42	57.33

Conclusion

It has been reported that ecological parameters such as humidity and temperature, greatly influence the disease development. The bacterial pathogen of *E. carotovora* sub sp. *Carotovora* was associated in the scorched tissue of cigar leaf in banana during the elevated temperature as the first report in the investigation due the stress caused by heat wave. To mitigate the abiotic stress by cultural method of planting of border crop with agathi *(Sesbania grandiflora)* tree and inter crop with dhaincha (*Sesbani aaculeate)* has significantly reduced the disease incidence by modifying the microclimate in the banana field.

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