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# Efficacy of Biocontrol Agents in Controlling Bacterial Wilt on Naga King Chilli (*Capsicum chinense* Jacq.)

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**Abstract**

***Available biocontrol agents were evaluated either alone or in various combinations for finding out their efficacy in suppressing bacterial wilt incidence and promoting plant growth of Naga king chilli (Capsicum chinense Jacq.) under field conditions. Among all tested combination, the treatment containing combination of T. viride +P. fluorescens was found most effective in reducing the incidence of bacterial wilt in field condition. Highest per plant yield was also recorded from the same combination and it was followed by T.viride. However, the commercially available fungicide Copper oxychloride (0.1%) showed the lowest (11.11%) disease incidence in field condition.***

***Key Words: Biological control, Naga king chilli, Bacterial wilt, T.viride, P. fluorescens***

## I. INTRODUCTION

Naga King Chilli (*Capsicum chinense* Jacq.) also known as Bhut Jolokia is the world's hottest chilli entered in the Guinness Book of World Record (2006). It is one of the important commercial spice crops of Assam. The crop has great market demand and fetches high price in both domestic as well as international market and generates high net return per unit area because of its high market price. Due to its extra-ordinary pungency level, oleoresin powder extracted from Naga king chilli is predicted to dominate the world market in coming years as the mainstay for riot control (Ritesh *et al*, 2000).

Naga king chilli suffers from many pest and diseases amongst which bacterial wilt disease of young plants caused by *Ralstonia solanacearum* (Yabuuchi et al., 1996) is one of the important limiting factor in cultivation and production of Naga king chilli. The first symptom of bacterial wilt starts with the wilting of the leaves. After a few days, a permanent wilt of the entire plant occurs, without exhibiting any symptom of leaf yellowing. *Ralstonia solanacearum* is a serious plant pathogen causing bacterial wilt in solanaceous vegetables in India. *Ralstonia solanacearum* gained its importance in the world scenery of pathology due to its severe destructive nature, wide host range and geographical distribution. The race 1, as reported by Denny (2006), is widely distributed in tropical and subtropical regions and it infects over 50 families including those in the Solanaceae i.e., eggplant (*Solanum melongena* (L.), pepper (*Capsicum* sp.), potato (*S. tuberosum* (L.) and tomato (*S. lycopersicum* (L.). Losses caused by the disease vary from 20-100%.

The bacterium *R. solanacearum* has been reported to be primarily a soil borne and water borne pathogen (Adebayo and Ekpo, 2005). It is a gram-negative, rod-shaped, largely aerobic bacterium that is 0.5-0.7 x 1.5-2.0 µm in size. Liquid and solid agar growth media are commonly used for culture. For most strains optimal growth temperature is between $30^0$ C and 35°C (Denny and Hayward, 2001). It infects host plants primarily through roots, entering through wounds formed by lateral root emergence or by damage caused by soil borne organisms (Adebayo and Ekpo, 2006). The bacterium can also enter plants through stem injuries caused by insects, mishandling or from mechanical damage. Once inside roots or stems, the bacterium colonizes the intercellular spaces of

the root cortex and vascular parenchyma, eventually entering the xylem vessels ( Vasses, Frey and Trigalet 1995). In the xylem vessels, the pathogen dissolves the cell walls and produce highly polymerized polysaccharides that increase the viscosity of the xylem resulting plugging of the vessels and finally occurrence of wilt of the plant. High atmospheric humidity further favours the development of the disease.

## II. MATERIALS AND METHODS

This study was conducted in the laboratories of Regional Agricultural Research Station, Diphu and farm of the Krishi Vigyan Kendra , Karbi Anglong during the year 2013-14 and 2014-15..

### A. Isolation and identification of pathogens

Diseased Bhut Jolokia plant parts and infested soils were collected from the farmers' fields in the Karbi Anglong district,where the disease was prevalent. Collection of samples with disease symptoms was made from the infected field to isolate causal organism. Infected stem of target plants was cut obliquely at the base and placed in sterile distilled water.The stem pieces showing milky white ooze in water was selected for isolation of the pathogen. Isolation of pathogen was done on Triphenyl tetrazolium chloride (TTC) Agar medium ( Kelman, 1954). Pure culture preservation was done in Nutrient agar slants for further study.
Identification of the pathogen was done by characterizing morphologically, physiologically, culturally and biochemically by following the guidelines described in the Bergey's Manual of systematic Bacteriology (Garrity G. 2001)

### B. Pathogenicity test

After isolation of the causal organisms the pathgenicity test was conducted on specific host through Koch's postulates (1882) by root inoculation technique (Winstead and Kleman,1952) under two different conditions viz., potted and field conditions for confirmation of the actual pathogen. A set of three seedlings were inoculated with sterile distilled water to serve as control. The plants were observed for the symptoms. Pathogenicity test was confirmed after Koch's postulation

### C. Collection of data on abiotic factors

Data on abiotic factors such as temperature, humidity and rainfall were collected for the entire period from Cloud Weather Observatory of Regional Agricultural Research Station, AAU, Diphu.

### D. Bioagents

Antagonists biocontrol agents Trichoderma viride, T. harzianum and Pseudomonas fluorescens were collected from Plant Pathology Laboratory, Assam Agricultural University, Jorhat and was tested against R. solanacearum in field condition.

### E. Preparation of talc-based formulation of fungal and bacterial bioagents

Culture of fungal cells were maintained on the potato Dextrose medium (PDA) and bacterial cell were maintained in nutrient agar ( NA) medium. Both Trichoderma spp. and fluorescent Pseudomonas were multiplied separately in talcum powder at 1:3 ratios (v/w). Polypropylene bags with 1 kg finely sieved talcum powder were taken for multiplication of both fungi and bacteria, there added 10 ml filter water and exposed to steam in autoclave at 100 0C. 1% of Carboxy methyl cellulose (CMC) solution , 1% of Manitol solution and 0.1% of Humic acid solution were added to the Polypropylene bags containing 1 kg of talcum powder at the time of inoculation of fungi or bacteria to hold it together. The mixture were incubated at 26 ± 20 for 24 hrs for multiplication of Pseudomonas fluorescent and Trichoderma spp respectively. After 24 h of incubation the packets

 

were shacked manually for uniform spread of antagonist in the talcum powder. This operation was repeated after 48h, 72 h, 96h, 120h, 144h of incubation, respectively. After 168 h ( 7 days) of incubation and continuous shaking the inoculated packets were brought out from the incubator and evaluated for presence of required population of the antagonists (Vidhyasekaran and Muthamilan,1995).

**F. Field Trials**

In order to study the efficacy of respected bio control agents against the Bacterial wilt pathogen, field trials were conducted at the farmers sick plot as well as experimental farm of Krishi Vigyan Kendra,Karbi Anglong AAU,Diphu during May-Oct for 2 consecutive years in 2013–14 and 2014-15. King Chilli , farmers variety 'Raja' were grown in the nursery and transplanted into the field 5 weeks after sowing. Each plot consist of 36 plants spaced 75 cm . Treatments were arranged in Split Plot Design with three replications. Chemical fungicide Copper oxychloride (0.1%) was used as the standard check.

**G. Treatments**

| | |
|---|---|
| T1 | Trichoderma viride |
| T2 | T. harzianum |
| T3 | Pseudomonas fluorescens |
| T4 | T. viride + P. fluorescens |
| T5 | T. harzianum+ P. fluorescens |
| T6 | Copper oxychloride (0.1%) |
| T7 | Control |

**H. Analysis of Data**

Data were recorded on Disease incidence (%) , Plant height (cm), Average fruit weight (g)/plant,Yield/plant(g), No. of fruits/plant of the bio formulation treated King Chilli plants. Appropriate statistical tools were used to analyze the data following the procedure described Gomez and Gomez (1984).

$$\text{Percent disease incidence} = \frac{\text{Number of infected plant}}{\text{Total number of plant}} \times 100$$

 

## III. RESULTS AND DISCUSSION

### A. Characteristics of the local strain of Ralstonia solanacearum

| Colony Character | Morphological character | Cultural Character | Pathological Test | Biochemical test |
|---|---|---|---|---|
| Light pink, opaque, circular, medium surface, entire margin with low convex elevation and size measuring ranging from 3.0mm to 4.0 mm in diameter. | Size: 1.2-1.3x 2.5-4)Mm<br>Shape:Rod shaped<br>Flagella No. one<br>Capsule: + ve<br>Spore: - ve | Gram negative,<br>Oxygene requirement Aerobic,<br>KOH test: +<br>Growth Character:filiform<br>Pigment production on King's B medium<br>- ve | THR: +<br>Potato soft rot : -ve<br>Pathogenicity Test : +ve | Catalase production: +ve<br>Starch hydrolysis:+ ve<br>Gelatin liquefication:+ ve<br>H2Sproduction: + ve<br>Levan Production: - ve<br>Nitrate reduction: +ve<br>Ammonia production: - ve<br>3-Ketolactose test: -ve<br>Glucose utilization: Oxidative<br>Arginine hydrolase : - ve<br>Gas production: -ve<br>Growth on 0.6% NaCl: Inhibited<br>Growth on 0.1% TTC: Slight |

\+ Positive
\- Negative

THR Tobacco hypersensitive Reaction
TTC Triphenyl tetrazolium chloride

### B. Meteorological Parameters

The pathological studies were carried out under outside environmental conditions during the period from May, 2014 to October, 2014 and May, 2015 to October 2015. The meteorological parameters recorded during the experimental period are presented in Table 1.

**Table 1. Meteorological Parameters during experimental period**

| Year | Month | Temperature (Max.)0C | Temperature (Min.) 0C | Relative Humidity | | Rainfall (mm) | Number of rainy days |
|---|---|---|---|---|---|---|---|
| | | | | Morning | Evening | | |
| 2014 | May | 33 | 23.2 | 94 | 57 | 91 | 7 |
| | June | 32 | 25.5 | 93 | 70 | 251.3 | 11 |
| | July | 32.8 | 25.8 | 96 | 75 | 248.5 | 13 |
| | August | 32 | 25.8 | 94 | 77 | 105.4 | 12 |
| | September | 31.8 | 24.6 | 94 | 76 | 144.5 | 11 |
| | October | 31.5 | 21.4 | 93 | 64 | 41.1 | 3 |
| 2015 | May | 31 | 22.3 | 92 | 61 | 139.3 | 13 |

 

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | June | 32.5 | 24.5 | 93 | 68 | 224.7 | 21 |
| | July | 33.4 | 25.6 | 93 | 72 | 141.5 | 12 |
| | August | 32.4 | 25.6 | 94 | 71 | 131.0 | 11 |
| | September | 32.8 | 25.0 | 93 | 72 | 159.8 | 10 |
| | October | 31.5 | 21.2 | 94 | 71 | 68 | 6 |

**B. Efficacy of antagonists on incidence of bacterial wilt under field condition**

In the field experiment (Table 2) all the treatments were found significantly superior to the absolute control in reducing the incidence of the bacterial wilt. The minimum disease incidence (20.37%) was recorded from the combination of T.viride and P.fluorescens application, although this was the second best treatment as compared to copper oxychloride (0.1%) application where the disease incidence was recorded as 11.11%.

In addition to reducing the disease incidence , the antagonist treatment T.viride + P. fluorescens also showed the superiority in field condition in respect of average plant height (81.17cm) and average fruit weight (7.27g/fruit) over the application of copper oxychloride. In copper oxychloride treatment plots the average height was found 80.81cm with average fruit weight of 7.05g/pt.

In respect of fruit yield, the chemical treatment copper oxychloride showed the highest fruit yield (831.83g/plant). Among the antagonist treatments, the highest fruit yield was recorded in T.viride + P. fluorescens (711.87g/pt) which was significantly different from control as well as all other antagonist treatments. The treatment T.viride was found second best among all the antagonist treatments in respect of yield (555.9g/pt), lower disease incidence (23.14%), plant height (79.45cm) and number of fruits per plant (79) while the treatment Pseudomonas fluorescens was occupied second position in respect of average fruit weight (7.22 g/fruit).

**Table 2. Effect of antagonists and chemical fungicide on the incidence of bacterial wilt and plant growth of chilli at 90 days after planting under field condition.**

| No. | Treatment | Disease Incidence (%) | Yield (g/plant) | Plant height (cm) | No. of fruit /plant | Average fruit weight (g) |
|---|---|---|---|---|---|---|
| 1. | Trichoderma viride | 23.14 (28.73) | 555.99 | 79.45 | 79 | 7.04 |
| 2. | Trichoderma harzianum | 30.55 (33.58) | 489.70 | 73.97 | 71 | 6.90 |
| 3. | Pseudomonas fluorescens | 25.59 (30.33) | 541.25 | 76.17 | 75 | 7.22 |
| 4. | T.viride + P.fluorescens | 20.37 (26.85) | 711.87 | 81.17 | 98 | 7.27 |
| 5. | T.harzianum+ P.fluorescens | 26.51 (30.98) | 503.50 | 75.15 | 73 | 6.90 |
| 6. | Copper oxychloride | 11.11 (19.46) | 831.83 | 80.81 | 118 | 7.05 |
| 7. | Control(Pathogen) | 41.50 (40.11) | 344.10 | 70.73 | 54 | 6.37 |
| | CD(5%) | 3.69 | 35.14 | 0.71 | 5.57 | 0.18 |
| | CD(1%) | 5.18 | 49.27 | 1.00 | 7.81 | 0.26 |

All values are means of three replications.
Figure in parenthesis indicates angular transformed values

 

Glick ( 1995) reported that plant growth rhizobacteria promote plant growth directly or indirectly, via production of phytohormone, biocontrol of host plant disease or improvement plant nutrient status. It is also evident from the present results that combination of *T.viride* + *P. fluorescens* is more effective against *Ralstonia solanacearum* causing bacterial wilt in Naga king chilli compared to individual antagonist

The results reveals the possibility of using eco friendly isolates of *Trichoderma viride* and *Pseudomonas fluorescens* for the management of bacterial wilt of Naga King Chilli.

## BIBLIOGRAPHY

[1] Anuratha C.S and Gnanamanickam S.S. (1990) Biological control of bacterial wilt caused by Pseudomonas solanacearuminIndia withantagonistic bacteria. Plant and Soil. 124: 109-116.

[2] Blair J.E., LenetteE.H. and TruantJ.P. (1971) In:Laboratory Exercise in Microbiology (Ed. M.J Pleczer and E.C Chan). Mc. Graw Hill BookCo., Berlin: p.356.

[3] Bora L.C and DekaS.N. (2007) Wilt disease suppression and yield enhancement in tomato (*Lycopersicon esculentum*) byapplication of *Pseudomonas fluorescens* Based biopesticide (Biofor-Pf) in Assam. Indian Journal of Agricultural Science. 77(8): 490-494.

[4] Buddenhagen I.W, Sequeira L. and Kelman A. (1962) Designation of races in *Pseudomonas solanacearum*. Phytopathology. 52: 726.

[5] Bull C.T, Weller D.M andThomashow L.S. (1991) Relationship between rootcolonization and Suppression Gaeumannomyces graminisvar. triticiby Pseudomonas fluorescens strain2-79. Phytopathology. 81: 954-959.

[6] BurrT.J, SchrothM.Nand SuslowT. (1978) Increased potato yields by treatment of seed pieces with specific strains of Pseudomonas fluorescensand Pseudomonas putida. Phytopathology. 68: 1377-1383.

[7] Dupler M. and Baker R. (1984) Survival of Pseudomonas putida, a biological control agent in soil. Phytopathology. 74:195-200.

[8] GarrityM.George (Eds.). (2001) Bergey's Manual of Systematic Bacteriology. Second Edition. Springer Verlag, New York.

[9] Glick BR (1995) The enhancement of plant growth by free living bacteria. Canadian Journal of Microbiology. 41:109 -117.

[10] Gomez KA, Gomez AA (1984) Statistical Productures for Agricultural Research.2nded. Wiley, New York, 680pp.

[11] Guinness book of world records (2006) Hottest spice. www.guinnessworldrecords.com. Accessed 2nd May 2007.

[12] Haas D.and DefagoG. (2005) Biological control of soil borne pathogens by fluorescent pseudomonas. Nature Reviews Microbiology. 3:307-319.

[13] Hayward A.C. (1964) Characteristics ofPseudomonas solanacearum.J. Appl. Bacteriol. 27: 265- 277.

[14] Hayward A.C.(1991) Biology andEpidemiologyofbacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev.Phytopathol. 29: 65-87.

[15] IslamTajulM.D and ToyotaKoki. (2004) Suppression of bacterial wilt of tomato by *Ralstonia solanacearum* by incorporation ofcomposts insoil and possible mechanisms. Microbes Environ. 19(1):53-60.

[16] Jinnah M.A, Khalequzzaman K.M, Islam M.S, Siddiqui M.A.K.S and AshrafuzzamanM. (2002) Control of bacterial wilt of tomato by Pseudomonas fluorescensin the field. Pakistan Journal of Biological Sciences. 5(11):1167-1169.

[17] Kelman A. and Person L.H. (1961) Strains of *P. solanacearum* differeingin pathogenicity totobacco and peanut. Phytopathology. 51: 158-161.

 