Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 25, No. (3) : 2023 : 529-534

ISSN-0972-3005
DOI No.: http://doi.org/10.53550/AJMBES.2023.v25i03.023

RECENT STRATEGIES FOR MANAGEMENT OF BASAL STEM ROT DISEASE IN COCONUT WITH INFLUENCE OF BACTERIAL ENDOPHYTE *BACILLUS AMYLOLIQUEFACIENS*

V. GOVARDHAN RAO[*1], B.NEERAJA[1], N.B.V. CHALAPATHI RAO[1], A. KIREETI[1], V. ANOOSHA[1], G. KOTESWARAO[1], VINAYAK HEGDE[3], RAVI BHAT[3], KIRAN KUMAR K.C. [2] AND B. SRINIVASULU[1]

[1]AICRP on Palms, Horticultural Research Station, Dr. Y.S.R. Horticultural University, Ambajipeta 533 214, Andhra Pradesh, India
[2]AICRP, HREC, Arsikere, Hassan 573 103, Karnataka India
[3]ICAR-Central Plantation Crops Research Institute, Kudlu, P.O, Kasaragod 671124, Kerala, India

(Received 25 March, 2023; Accepted 13 May, 2023)

Key words: *Basal stem rot, Coconut, Endophyte, Biocontrol, Bacillus amyloliquefaciens, Ganoderma*

Abstract– Coconut palm in spite of its hardiness is affected by a large number of diseases, among which Basal Stem Rot (BSR) disease caused by *Ganoderma lucidum* (Leys) Karst.and *Ganoderma applanaum* are the most destructive and a major limiting factor in coconut production. In the present study, endophytic bacteria EP4 isolated from the roots of healthy coconut palms was identified as *Bacillus amyloliquefaciens* and showed promising inhibition by 51.11% over control when it was studied under in vitro conditions against test pathogen. Further, when it was applied in the basin of Basal stem rot affected coconut palm @100g talc formulation of *Bacillus amyloliquefaciens* along with 50kg FYM and subsequent application in quarterly interval with only talc formulation of endophyte, over a period of 30 MAT, it showed significant inhibition of 23.33% over initial which was also on par with root feeding of the same@50 ml broth (10^8 cfu/ml) in 100 ml water recorded 22.12 % reduction over initial. However, the positive check with root feeding of Hexaconazole @ 3 ml in 100 ml water given in quarterly interval showed 39% disease reduction over initial.

INTRODUCTION

Coconut palm (*Cocos nucifera*, L.) is an important plantation crop of India and often described as 'Kalpavriksha' because of the multifarious uses of every part of it in the commercial sector. Coconut palms are successfully grown in the tropical countries of the world and are hence referred to as "King of the tropical palms." The South Pacific and South Africa are often cited as possible center of origin. Coconut provides food, drink, shelter and industrial raw materials. Coconut is grown in almost 94 countries in the world of which 90% of the production comes from Asian and Pacific countries.

Coconut plays vital role in rural horticulture economy with a total Production of 13411 T tonnes in India and 1112T tonnes as share of 8.27% and ranks 4th in Andhra Pradesh with a area and production (1.15 lakh ha; 1378 million nuts) after Kerala, Karnataka and Tamil Nadu with a productivity of 11957 nuts/ ha 2021-22). In Andhra Pradesh, East Godavari (50,490 ha), West Godavari (21, 818 ha), Srikakulam (14,753 ha) and Visakhapatnam (7300 ha) districts occupy major area in forefront in coconut cultivation.

Coconut palm in spite of its hardiness is affected by a large number of diseases, among which basal stem rot (BSR) disease caused by *Ganoderma lucidum* (Leys) Karst and *Ganoderma applanaum* are the most destructive and a major limiting factor in coconut production especially in Tamil Nadu, Andhra Pradesh and other coconut growing states of India (Bhaskaran *et al*., 1989). Currently, no cost-effective fungicide is available that gives guaranteed control. Development of biological control for basal stem rot disease is accepted as a durable and environment friendly alternative for agrochemicals. In biocontrol methods, the use of endophytic bacteria is emphasized by many authors. Endophytic bacteria have been shown to control *Fusarium oxysporum* f.

sp. *vasinfectum* on cotton (Chen *et al*.,1995). Garrett (1955) reported that *Trichoderma viride* and *Streptomyces* spp. were antagonistic to *G. lucidum*. Soil application of *T. viride* and Pseudomonas *fluorescens* talc formulations at the rate of 200 g each/ palm in combination with 50 kg FYM was found effective against the disease (Karthikeyan et al. 2005). Baker and Cook (1974) suggested that antagonists which differed in their ecology could be combined so that they could effectively utilise the root exudates and survive in association. With this background, the present study was undertaken to see the effect of bioagents individually and in combination against Ganoderma spp. *in vitro.*

MATERIALS AND METHODS

Isolation of coconut pathogens: The Coconut palm depicting characteristic symptoms of basal stem rot disease was selected and Infected roots/ stem bits collected from infected palms were washed thoroughly with sterile water and cut into small bits/pieces and were surface sterilized in 1 per cent sodium hypochlorite solution for 30 seconds and rinsed with sterile distilled water thrice serially to remove the traces of sodium hypochlorite. After surface sterilization, diseased specimens were kept in sterilized bags along with wet cotton under room temperature for about 8 to10 days. After 8 to 10 days of incubation period, slight mycelial growth was observed and that was transferred into potato dextrose agar (PDA) medium. The inoculated plates were incubated at room temperature (28 °C ± 2 °C) for 3-5 days to facilitate growth of the fungus. Later, the bit of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by following hyphal tip culture technique under aseptic conditions [Plate No.1&2]

Isolation, Characterization and selection of effective endophytes associated with apparently healthy palms: Isolation, identification and Characterization of effective antagonistic endophyte from rhizospheric region of coconut was done as per standard protocol (Rajendran *et al.,* 2007).

In vitro testing of endophytic bacterial strains for inhibition of mycelial growth of Ganoderma spp: Bacterial endophytic strains were tested for their inhibition of mycelial growth of *Ganoderma lucidum* by following the dual culture technique (Dennis and Webster, 1971). The bacterial culture was streaked at one side of Petri dish (1 cm from the edge of a plate) with PDA medium and mycelial disc (8 mm diameter) of seven days old culture of *Ganoderma lucidum* was placed on the opposite side in the Petri dish perpendicular to the bacterial streak. The plates were incubated at room temperature (28 ± 2 °C) for four days and the mycelial inhibition of pathogen was measured in millimetre[Plate No.3&4]

Preparation of substrate for the multiplication of endophyte: The organic substrate of dried cow dung was tested for the multiplication of bacterial endophyte. One hundred gram of substrate was weighed in polypropylene bag, moisture content was adjusted to 60% (w/v) by gravimetric method (Dutta and Das, 1999). One set of treatments of the above substrate were sterilized and another set was kept without sterilization. For sterilization the substrates were autoclaved at 15lb psi for 1h on two consecutive days. The substrate was inoculated with 5 g of talc based bioformulation of bacterial endophyte. The substrate was incubated at room temperature.

Assessment of endophyte population in farm yard manure: One gram of sample from FYM was derived at regular intervals, and the population (CFU) of bacterial endophyte in the substrate and talc were counted at 10, 20, 30, 40, 50, 60 days intervals after inoculation by serial dilution techniques in selective medium for bacterial endophyte.

Field experiment: The bacterial bioagent which was found to be promising in arresting the growth of *Ganoderma* sp. under in vitro conditions. The same isolate was taken to field conditions to test field efficacy of *Bacillus amyloliquefaciens* against Basal stem rot disease in coconut. The talc based formulation was applied in soil along with FYM 50 Kg, where as bacterial broth was fed through roots and root feeding of Hexaconazole was used as standard check.

Tr.No.	**Treatment**
T1	Soil application of 100g talc formulation of *Bacillus amyloliquefaciens* along with 50kg FYM
T2	Root feeding of bacterial strain 50 ml broth (10^8 cfu/ml) in 100 ml water
T3	Root feeding of Hexaconazole 3 ml in 100 ml water

T4 Control

Statistical design –RBD, No of Treatments-4, Replications-6, Treatment interval-Quarterly. Observations recorded-Disease index

Disease index: All the experimental palms in this trial were indexed for disease using formula Disease index= 23.6+17.4H+36.6R-0.6L where H is height of bleeding patch, R- reduction in leaf size (0-4 scale), L-Number of functional leaves. The disease index at the beginning and at quarterly interval was recorded in individual replication and the average was calculated.

Statistical analysis: The data were statistically analyzed using the OPSTAT 1996 developed by O. P. Sheoran, "Hisar. Statistical Package for Agricultural Scientists (OPSTAT)," CCS HAU.

RESULTS AND DISCUSSION

Basal stem rot causing pathogen Ganoderma was isolates from the infected roots and sporophore.It was identified by cultural and morphological studies and it was confirmed as *Ganoderma lucidum* .The same pathogen was used as test pathogen for screening the Bacterial endophytes viz., EP1,EP2, EP3 and EP4 isolated from healthy coconut plant materials. Among four endophytes tested only EP4 was fond effective which showed 51.11 % inhibition over control. The promising endophyte isolate EP4 was further screened against various isolates of *Ganoderma* collected in Andhra Pradesh (Table 1).

Based on dual culture studies with promising endophytic bacteria was further screened against seven Ganoderma isolates collected from various locations of Andhra Pradesh and it was observed that on an average , more than 50% of inhibition over control was recorded. Hence the promising endophytic bacterium EP4 was selected for field experiment against Basal stem rot disease (Table 2). The promising endophyte EP4 was sequenced on outsourcing basis and identified it as *Bacillus amyloliquefaciens*.

>Coconut_Endophyte *Bacillus amyloliquefaciens*

TGCTATACATGCAAGTCGAGCGGACAGAT
GGGAGCTTGCTCCCTGATGTTAGCGGCG
GACG GGTGAGTA ACACG TGGGTAACCTG

Table 2. Evalution of endophyte EP4 against various Ganoderma isolates collected in Andhra Prdesh

S. No	Treatment	Mycelial growth (mm) of Ganoderma	Percent inhibition over control
T1	G1 × EP4	44.00(39.94)	51.11
T2	G2 × EP4	40.00(38.92)	55.56
T3	G3 × EP4	46.67(41.62)	48.15
T4	G4 × EP4	41.67(39.55)	53.70
T5	G5 × EP4	43.33(40.27)	51.85
T6	G6 × EP4	37.33(37.20)	58.52
T7	G7 × EP4	33.33(34.78)	62.96
T8	Control	90.00(71.53)	0.00
	CD@1%	1.177	
	SEm+	0.397	

CCTGTAAGACTGGGATAACTCCGGGAAA
CCGGGGCTAATACCGGATGGTTGTCTGA AC
CGCATGGTTCAGACATAAAAGGTGGC
TTCGGCTACCACT TACAGATGGACCCG CGG
CGCATTA GCTAGTT GGTGAG GTAACGGCT
CACC AAGG CGACGAT GCGTAGC CGACCTGA
GA GGGTGA TCGGCC ACAC TGGG ACTGAGA
CACGGCCCAGACTCCTACGGGAGGCAG CAG
T A G G G A A T C T T C C G C A A T G G A C G
AAAGTCTGACGGAGCAACGCCGCGTGAGT
GATGAAGGTTTTCGGATCGTAAAGCTCTG
TTGTTAGGGAAGAACAAGTGCCGTTCAA
ATAGGGCGGCACCTTG ACGGTACCTAACCA
GAAAGCCACGGCTA ACTACGTG CCAGCAGC
CGCGGT AATACGTAGG TGGCAAGCG TTGTCC
GG AATTATTGGG CGTAAA GGGCTC GCAGG
CGG TTTCTTAAG TCTG ATGTGAA AGCC
CCCGGC TCAACCGG GGAGGGTCATTGGA
AACTG GGGAACTTG AGTGCAGAAGA GGAGA
GTGGAATTCCAC GTGTAGCGGTG AAATG
CGTA GAGATGTGGAGGAA CACCAGT GGCGA

Table 1. Preliminary screening of Endophytes against test pathogen under in vitro conditions

Sl. No.	Dual culture study: Ganoderma x Endophyte	Mycelial growth (mm) of Ganoderma at 8 DAI	Percent inhibition over control
T1	*Ganoderma lucidum*-Control	90.00(69.31)	0.00
T2	*Ganoderma* x EP1	85.00(66.89)	5.56
T3	*Ganoderma* x EP2	90.00(67.15)	0.00
T4	*Ganoderma* x EP3	90.00(68.04)	0.00
T5	*Ganoderma* x EP4	44.00(39.11)	51.11
	CD@1%	3.99	
	SEm±	1.31	

DAI- Days after inoculation

AGGCG ACTCTCTGGTCT GTAACT GACGC TGAGGAGCGAAAGC GTGGGGAGCGAAC AGGATTAG ATACCCTG GTAGT CCACGCCGTA AACGATGAGTGCTAAGTGTTAGGGG GTTTCCG CCCCTTAGTGCTG AGCTAACGCATTAAGCAC TCCGCC TGGGGAGTACGGTCG CAAGACTGA AACTCA AAGGAATTGACGGGGGC CCGCACA AGCGGTGGAGCATGTGGTTTAATTC GAAGCAACGCGAAGvAACCTTACCAGG TCTTG ACATCCTCTGACAATCCTAGAGATA GGACGTCCCCTTCGGGGGCAGAGT GACAGGTGGTGCATGGTTGTCGTCAGC TCGTGTCGTGAGATGTTGGGTTAAG TCCCGC AACGAGCGCAACCCTTGATCTTAGTTGCC AGCATTCAGTTGGGCACTCTAAGGTGAC TGCC GG TGACAAACCGGAGGAAGGTG GGGA TGA CGTCAAATCATCATGCCCCTT ATGACCT GGG CTACACACGTGCTACAAT GGACAGAACAA AGGGCAGCGAAACCGCGAGGTTA AGCCAATCCCACAAATCTGTTC TCAG TTCGG A TCGCAGTCTGCAACTCG ACTGCG TGAAG CT GGAATCGCTAGTAA TCGCGGATCA GCA TGC CGCGGTGAATACGT TCCCG GGCC TTGT ACAC ACCG CCCGTCA CACCA CGAG AGTTTGTA ACACCCG AAGTC GGTGA GG TAACCTTTAT GGAGCC AGCCGCC GAAG.

Field experiment: The bacterial endophyte used as bioagent named *Bacillus amyloliquefaciens* was found to be promising in arresting the growth of *Ganoderma* sp. under in vitro conditions. The same isolate was taken to field conditions to test field efficacy of *Bacillus amyloliquefaciens* against Basal stem rot disease in coconut. It was observed that, during initial nine months there was no difference among the treatments imposed with respect to disease index. However, after nine months, it was showed significant variation with respect to disease suppression (Table 3).

T_1- Soil application of 100g talc formulation of *Bacillus amyloliquefaciens* along with 50kg FYM

T_2 -Root feeding of bacterial strain 50 ml broth (10^8 cfu/ml) in 100 ml water

T_3 - Root feeding of Hexaconazole 3 ml in 100 ml water

T_4 – Control

The results at 30 months after treatment T1- Soil application of 100g talc formulation of *Bacillus amyloliquefaciens* along with 50kg FYM found superior with 23.33 % reduction over control and was on par with T2 -Root feeding of bacterial strain @ 50 ml broth (108 cfu/ml) in 100 ml water recorded 22.12 per cent reduction over initial. Further the positive check recorded 39.00 % of reduction over initial (Table 3 and Plate 5).

DISCUSSION

In the present study, endophytic bacteria EP4 isolated from the roots of healthy coconut palms showed promising inhibition by 51.11% over control when it was studied under in vitro conditions against test pathogen. Further, when it was applied in the basin of Basal stem rot affected coconut @100g talc formulation of *Bacillus amyloliquefaciens* along with 50kg FYM, it was showed significant inhibition of 23.33% over initial which was also on par with root feeding of the same@50 ml broth (10^8 cfu/ml) in 100 ml water recorded 22.12 % reduction over initial. The results are in conformity with Hallman *et al.* (1997) reported that most of the endophytic bacterial strains are capable of promoting plant growth. Endophytic bacteria colonize a broad spectrum of plant species and plant parts (Sturz *et al*., 1997). *Bacillus* species are among the most common bacteria found to colonize plants endophytically (Mahaffee and Kloepper 1997). Bhowmik *et al*. (2002) reported that seed bacterization with endophyte, Endo PR8 was found to be the most effective to reduce he cotyledonary infection by Xam. The endophytic Bacillus spp. CY22 isolated from balloon flower produced iturin A with antifungal activity against *Rhizoctonia solani, Pythium ultimum and Fusarium oxysporum* (Cho *et al.,* 2003). Endophytic bacteria viz., *B. amyloliquefaciens* produces surfactin, iturin, bacillomucine, azalomycin F, *B. Subtilis produces* surfactin and arthrobactin and *B. pumilus* produces surfactin, amphomycin, arthrobactin and valinomycin which are effective against black rot of crucifers caused by *X. campestris pv. campestris* (Monteiro *et al*., 2005). Similar efficacy may be expressed by the EP4 when soil application along with FYM resulted suppression of the soilborne pathogen *G. lucidum*. Their maximum temperature for growth is in the range 30–40 °C (Rifai, 1969).

Closest match identified was *Bacillus amyloliquefaciens*

Isaolates name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Coconut_endo_1432	2645	2645	100%	0.0	100.00%	MK215647.1

Table 3. Influence of bacterial bio-agents on Basal stem rot disease of coconut :

Treatments		Disease index										reduction
	Before treatment	3 MAT	6 Mat	9 MAT	12 Mat	15 MAT	18 MAT	21 MAT	24 MAT	27 MAT	30 MAT	Over initial
T_1	38.48	38.48	38.09	38.48	36.77	35.22	33.15	32.48	30.50	30.01	29.54	23.33
	(38.28)	(38.28)	(38.05)	(38.28)	(31.07)	(36.40)	(35.15)	(28.81)	(29.20)	(33.12)	(32.84)	
T_2	34.69	34.69	34.69	33.26	30.21	27.73	27.52	27.24	27.86	27.20	27.01	22.12
	(35.68)	(35.68)	(35.68)	(34.83)	(27.84)	(31.78)	(31.64)	(31.47)	(31.21)	(31.22)	(31.10)	
T_3	41.46(39.88)	42.25	42.25	42.25	39.43	34.76	29.35	27.15	26.29	25.29	25.29	39.00
		(40.39)	(40.37)	(40.39)	(32.19)	(36.13)	(28.36)	(25.77)	(25.53)	(30.04)	(30.04)	
T_4 –	Control	52.37	53.54	54.25	54.32	55.43	55.78	57.53	59.24	61.32	61.32	61.32
		(49.57)	(50.25)	(50.07)	(50.66)	(40.03)	(48.32)	(41.04)	(42.60)	(42.91)	(51.55)	(51.55)
	S.Em±	6.14	6.01	5.75	5.60	1.19	1.20	1.45	1.96	1.93	1.72	1.67
	CD (P=0.05)	**NS**	**NS**	**17.73**	**17.27**	**3.59**	**3.63**	4.39	5.92	5.84	5.23	5.10

Plate 1. Symptoms of Basal Stem Rot

CONCLUSION

Coconut palm is infected by *Ganoderma spp* are causing Basal stem rot also called as Ganoderma wilt in coconut. It was the most destructive and a major limiting factor in coconut production. In the present study, endophytic bacteria EP4 identified as *Bacillus amyloliquefaciens* showed promising inhibition against Ganoderma under *in vitro*. Further, when it was applied in the field against Basal stem rot affected coconut palm, it also showed significant inhibition compared to control. Though it is inferior to the chemical control but it is eco friendly and sustainable approach to manage the disease. Hence, it may be suggested to use as biocontrol of basal stem rot disease in coconut.

ACKNOWLEDGEMENT

I should convey my heartfelt thanks to the Project co ordinator, ICAR-AICRP on Palms and ICAR-CPCRI, Kasagod, Kerala for financial support and technical supported rendered towards successful execution of this work at Dr.YSRHU-HRS, Ambajipeta. I also extend my sincere thanks to Principal scientist and Head, Dr. YSRHU-HRS, Ambajipeta for the reinforcement and encouragement for implementing the technical programmes in timely.

REFERENCES

Baker, K.F. and Cook, R.J. 1974. *Biological Control of Plant Pathogens*. W.H. Freeman and Co., San Francisco, 433 pp

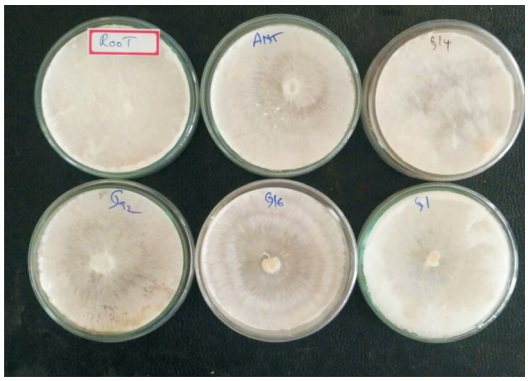

Plate 2. *Ganoderma* isolated from root and bark samples

Plate 3. Isolation and selection of endophytes associated with apparently health palms

Plate 4. Dual culture by using endophytes against Ganoderma

Plate 5. Soil application of 100g talc formulation of Bacillus amyloliquefaciens along with 50kg FYM

Bhaskaran, R., Rethinam, P. and Nambiar, K.K.N. 1989. Thanjavur wilt of coconut. *J. Plant. Crops*. 17: 69–79.

Bhowmik, B., Singh, R.P., Jayaram, J., Verma, J.P. 2002. Population dynamics of cotton endophytic Pseudomonas, their antagonism and protective action against the major pathogens of cotton. *Indian Phytopath.* 55 (2): 124–132.

Chen, C., Bauske, E.M., Musson, G., Rodriguez-Kabana, R. and Kloepper, J.W. 1995. Biological control of *Fusarium* wilt on cotton by use of endophytic bacteria. *Biol. Control.* 5: 83–91.

Cho, S.J., Lim, W.J., Hong, S.Y., Park, S.R. and Yun, H.D. 2003. Endophytic colonization of balloon flower by antifungal strain *Bacillus* sp. CY22. *Biosci. Biotechnol. Biochem*. 67 (10): 2132–2138.

Denis, C. and Webster, J. 1971. Antagonistic properties of species growth of Trichoderma-III. Hyphal interaction. *Trans Br.Mycol. Soc*. 57: 363-369.

Dutta, P. and Das, B.C. 1999. Effect of seed pelleting and soil application of *Trichoderma harzianum* in the management of stem rot of soybean. *J. Mycol. Pl. Pathol*. 29 (3): 317–322.

Garrett, S.D. 1955. A century of root disease investigation. *Ann. Appl. Biol*. 42: 211–219.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W.F. and Kloepper, J.W. 1997. Bacterial endophytes in agricultural crops. *Can. J. Microbiol*. 43: 895–91

Karthikeyan, G., Karpagavalli, S., Rabindran, R. and Natarajan, C. 2005. Biological control of basal stem rot disease in coconut. *The Planter*. 81 (957): 777–784.

Mahafee, W.F. and Kloepper, J.W. 1997. Temporal changes in the bacterial communities of soil, rhizosphere and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). *Microbial. Ecol.* 34: 210–223

Monteiro, L., Mariano, R.L. and Souto-Maior, A.M. 2005. Antagonism of *Bacillus* spp. against *Xanthomonas campestris* pv. campestris. *Braz. Arch. Biol. Technol*. 48: 23–29

Rajendran Lingan, Karthikeyan Gandhi, Thiruvengadam Raguchander and Ramasamy Samiyappan, 2007. *In vitro* evaluation of bacterial Endophytes influence on *Ganoderma lucidum* (leys) karst. Mycelial growth. *Journal of Plant Protection Research*. 47(4).

Rifai, M.A. 1969. A revision of the genus Trichoderma. *Micol. Papers.* 116: 1–56.

Sturz, A.V., Christie, H.R., Matheson, B.G., Arsenault, W.J. and Buchman, N.A. 1997. Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol. Fert. Soils*. 25: 13–19.