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Efficacy of Fungal and Bacterial Bio-control Agents on *Ganoderma* Spp. Causing Foot Rot of Arecanut

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***Abstract* – Fungal and bacterial bio-control agents were evaluated against *Ganoderma* sp., the causal Foot rot disease of Arecanut under laboratory conditions at the Department of Plant Pathology, University of Agricultural Sciences, GKNK, Bengaluru to identify the potential Bio-agents under *in vitro* conditions. The fungal bioagent *Trichoderma koningi* (Tk-1) recorded maximum (59.86) inhibition of the test fungus and remained *on par* with *T. viride* (Tv-GKVK2). However, the volatile metabolites of *T. viride* (Tv - NBAIR) recorded maximum (71.07%) inhibition of the test fungus but remained *on par* with *T. harzianum* (Th - NBAIR), *T. viride* (Tv -AMB) and *T. viride* (Tv-GKVK1). Among the bacterial bio-agents, *B. amyloliquefaciens* (Ba-43) recorded maximum (63.93 %) inhibition followed by *B. subtilis* (Bs-P22) which accounted 59.27 per cent inhibition over control in dual culture technique. When the effect of volatile metabolites of bacterial bioagents considered, *B. subtilis* (Bs-P22) recorded maximum (70.53 %) inhibition followed by *B. subtilis* (Bs-P42) and *P. putida* (Pp-1) which accounted 70.00 and 67.07 per cent inhibition over control by producing volatile metabolites. The volatile metabolites of Fungal and bacterial bioagents are found to more promising in inhibition of test fungi compared to the dual culture technique under *in vitro*.**

***Keywords* – Arecanut, Foot Rot, *Ganoderma,* Bioagents, Volatile Metabolites.**

I. Introduction

Arecanut is a tropical plant found all over South-East Asia. The fruit (nut) of this tree is popularly known as the betel nut or supari in India. This is an important commercial crop of the region and also forms part of ritual offerings in hindu religion. Areca is taken up from the Malayan language which means ‘cluster of nuts'. The current production of arecanut in the world is about 127 thousand tonnes from an area of 925 thousand ha. India ranks first in both area (49 %) and production (50 %) of arecanut. Other major arecanut producing countries are Indonesia (16 % area and 15 % production), China (5 % area and 11 % production) and Bangladesh (20 % area and 8 % production). In India, arecanut is cultivated in an area of 4.53 lakh hectares with an annual production of 6.32 lakh tonnes. In India, arecanut is popular for masticatory purpose and is chewed either with betel leaves or as scented supari. Arecanut cultivation is concentrated in South western and North eastern regions. The major states cultivating this crop are Karnataka (40 %), Kerala (25 %), Assam (20 %), Tamil Nadu, Meghalaya and West Bengal which accounts for more than 70 per cent of the area and production.

Arecanut palms are normally affected by various biotic and abiotic stresses resulting in drastic reduction in yields. Among the various biotic stress that affect arecanut production in India. Foot rot of areca commonly known as Anabe roga in Kannada is caused by *Ganoderma lucidum*, which is a soil borne braket forming fungus is a major constraint in arecanut production, especially in dry tracts of Southern Karnataka. Coleman reported foot rot from Karnataka during 1911. The disease causes gradual decline of the palms and kill them slowly. The pathogenic nature of *Ganoderma lucidum* was established in early 1990 by artificial inoculation on to live palms [1]. *Ganoderma* wilt (BSR) of palms is being contained by following integrated disease management practices. Although several workers [1, 2, 3 & 4] have reported different management practices against *Ganoderma* wilt of coconut and arecanut, the results were inconsistent and not much work has been done relating to the efficacy of bio-control agents especially volatile metabolites of BCAs on *Ganoderma* spp. causing Foot rot in Arecanut. Hence, present study was carried out to evaluate efficacy of fungal and bacterial bio-agents against *Ganoderam* Spp. causing Foot rot disease in Arecanut under *in vitro* conditions, which intern it will be useful for further integration and investigation in the integrated disease management practices under field conditions to combat deadly disease of arecanut.

II. Materials and Methods

Infected roots/ stem bits and sporocaps collected from infected palms of arecanut were washed thoroughly with sterile water and cut into small bits/pieces and were surface sterilized in 0.1 per cent mercuric chloride for 30 seconds and washed three times serially in sterile distilled water to remove the traces of mercuric chloride. After surface sterilization diseased specimens were kept in sterilized bags along with wet cotton under room temperature for about 8 to 10 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. The isolated pathogen was identified based on colony and mycelial characteristics. The isolated fungus was sub-cultured on PDA slants and allowed to grow at 28 °C ± 2°C temperature for 8-10 days.

a) Dual Culture Technique

The efficacy of ten fungal and eight bacterial antagonists were evaluated against *Ganoderma* isolate of arecanut (AG_7) for radial growth inhibition on the potato

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dextrose agar medium using dual culture technique under *in vitro* condition (Table I). Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and was allowed to solidify. For evaluation of fungal bio-control agents, mycelial discs of test fungus were inoculated at one end of the Petri plate and antagonistic fungus was placed opposite to it on the other end. Observations were recorded on mycelia growth of fungus up to seven days. For each treatment three replications were maintained. In case of evaluation of bacterial antagonist, the bacterium was streaked at one end of the Petri plate and the test fungus was placed at the other end. The plates were incubated at 28 ± 2 °C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent [5]

$$I = 100 (C-T) / C$$

Where,
I = Per cent inhibition of mycelium.
C = Growth of mycelium in control.
T = Growth of mycelium in treatment.

b) Production of Volatile Compounds

The efficacy of ten fungal and eight bacterial antagonists were evaluated against *Ganoderma* isolate of arecanut (AG_7) for radial growth inhibition on the potato dextrose agar medium to test inhibitory effect of volatile compounds on test fungus under *in vitro* condition. For this study twenty ml potato dextrose agar was poured onto each Petri dish. A 6 mm diameter agar disc excised from the leading edge of two days old pure culture of each of fungal bio control agent cultures was placed at the centre of each agar plate. Similarly, bacterial bio control agents were streaked on previously poured petri dish containing nutrient agar. Afterwards, a disc of the same size was taken from *Ganoderma* isolate of arecanut (AG_7) culture, likewise placed on another agar plate. The lids were removed, and test fungus culture plate was immediately placed over each of bio control agents plates, held together with adhesive tapes. The head space prevented any physical contact between the causal pathogen and antagonistic fungi, so that the volatile compounds were formed and confined to the interior atmosphere of the two plates. For the control plate, only *Ganoderma* isolate of arecanut (AG_7) was cultured on each Petri dish. The plates were randomized and incubated at 28 ± 2 °C for 7 days. The diameters of test fungus colony cultures were measured daily. Three replicate plates were done for each treatment, incubated under room temperature conditions (28 ± 2 °C). The implication of results was expressed as percent inhibition over control as the dual culture experiments.

III. RESULTS AND DISCUSSION

The competitive ability of antagonists against *Ganoderma* isolate of arecanut revealed that, among ten strains/ species of fungal bio control agents *T. koningi* (Tk-1) recorded maximum (59.86) inhibition of the test fungus and remains on par with *T. viride* (Tv - GKVK2) which accounted 55.78 per cent inhibition over control under dual culture technique. However, when efficacy of volatile metabolites was considered *T. viride* (Tv - NBAIR) recorded maximum (71.07 %) inhibition of test fungus but remains on par with *T. harzianum* (Th - NBAIR), *T. viride* (Tv - AMB) and *T. viride* (Tv - GKVK1). Among the bacterial bioagents, *B. amyloliquefaciens* (Ba - 43) was recorded maximum (63.93 %) inhibition followed by *B. subtilis* (Bs - P22) which accounted 59.27 per cent inhibition over control in dual culture technique. When the effect of volatile metabolites of bacterial bioagents is considered, *B. subtilis* (Bs - P22) was recorded maximum (70.53 %) inhibition followed by *B. subtilis* (Bs - P42) and *P. putida* (Pp - 1) which accounted 70.00 and 67.07 per cent inhibition over control by producing volatile metabolites (Table. II, Fig. I and Plate I).

The present findings are in agreement with [6] who reported that, reported that native BCAs (*T. viride, T. harzianum* and *T. hamatum)* were effective in controlling basal stem rot pathogens (*G. lucidum* and *G. applanatum*) *in vitro*.

T. harzianum and *T. viride* were reported to be antagonistic to *G. lucidum* [7 & 8]. Bhansali [9] reported that in dual culture technique *Trichoderma* was found to inhibit the mycelial growth of *Ganoderma lucidum* on potato dextrose agar under *in vitro* conditions. Among the three strains of *T. harzianum* and *T. viride* inhibited the maximum mycelial growth by acting as antagonists to *G. lucidum*. Iyer *et al.* [10] reported that four fungal cultures viz., an unidentified sterile white fungus (77.80), *Trichoderma harzianum* (72.20), *T. viride* (62.0) and *Pencillium* sp. (42.0) were found to be inhibitory on the mycelial growth of pathogen at 96 hrs. Similar observations were also made by Chakrabarty and Ray [11] who reported that, three fungal cultures viz., *Trichoderma harzianum* (63.99 %), *Trichoderma viride* (66.55 %) and *Gliocladium virens* (62.12 %) have inhibitory effect on the mycelial growth of the pathogen after 96 hours of incubation. Rajendran *et al.* [12] reported that endophytic strains of bacteria isolated from coconut roots of different regions effectively inhibited the *G. lucidum* growth *in vitro*.

Modh Zainudin and Abdullah [13] reported that disease suppression in *Ganoderma*-infected oil palm seedlings treated with a conidial suspension of *Trichoderma harzianum* FA 1132 was tested in plant house conditions to determine the effectiveness of the fungus as a bio control agent. Volatile Organic Compounds (VOC) produced by microorganisms played an important role during their evolution in the context of their interactions, community population and functional dynamics. Such interactions will result in functional responses by the organisms involved to some community members and coincidental disadvantage to others. The substrate - dependent variation in VOC production will result in variations in microbial and consequently systemic respons

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-e [14].

Several studies were carried out on the biological control of *Ganoderma* associated with oil palm basal stem rot (BSR) and its mode of action. The growth of *G. boninense* was inhibited when exposed to the trapped head space of volatile compounds produced in the presence of *T. harzianum* isolates. The inhibition started after 24 hours and increased until end of the experiment. Inhibitory effect on the presence of head space gases on agar petri dishes indicated that the unidentified volatile substances suppressed the pathogenic fungus of *G. boninense*. These results are consistent with the study conducted by Mumpuni *et al.,* [15], that the headspace gases generated by *Rhizoctonia solani* inhibited *A. bisporus* growth to a lesser extent than *T. harzianum* (strains: Th1 and Th2). Doi and Mori [16] also observed the volatile compounds from *Trichoderma* spp. impeded the hyphal growth of different fungal pathogens on agar plates. They reported that *T. viride* produced large amount volatile compounds to affect the hyphal tips of *Lentinus lepidus* and *Coriolus versicolor*. Gary *et al.* [17] (2001) identified five classes of volatile compounds, such as alcohols, esters, ketones, acids and lipids, produced by some fungi and bacteria.

All of the isolates of *T. harzianum* inhibited the growth of *Ganoderma boninense* (PER 71) with percentage of inhibition radial growth (PIRG) values ranging from 47.86 to 72.06 per cent. The best eight samples from the dual cultures were then tested for their production of volatile antifungal compounds against PER 71, which gave PIRG values between 24.53 and 58.70 per cent over 6 days. The values ranged from 18.35 to 40.16 per cent over 6 days for the antifungal activities of their non-volatile compounds. Isolate FA 30 was demonstrated to be the best isolate not only to the dual culture inhibition tests but also the best for the production of inhibitory properties from both volatile and non-volatile antifungal compounds [18].

Trichoderma harzianum has the ability to produce volatile and antifungal compounds [15] as well as non-volatile compounds particularly in the growth inhibition of *G. boninense* in *in vitro* studies [19]. One noted well-known volatile antifungal compound is a 6-pentyl-alpha-pyrone (6 PAP) which has been described as a secondary metabolite [20].

Zou *et al*. [21], found 38 volatile compounds produced by isolated soil bacteria. Twenty-nine commercial compounds with the same chemical structure as the bacterial volatiles were tested. Among them, acetamide, benzaldehyde, benzothiazole, 1-butamine, methanamine, phenyl acetaldehyde and 1-decene showed antifungal activity against *Paecillomyces lilacinus* and *Pochonia chlamydosporia*. Arrebola *et al.* [22], found that the major VOC molecules produced by *Bacillus subtillis* (46 %) and *B. amyloliquefaciens* (97 %) with fungicidal activity were ketones. Although soil fungi and bacteria are known to produce VOCs [23], their VOC antagonistic to plant-pathogenic microorganisms are rarely studied which needs to be expanded due to a variety of soil types and conditions in world agriculture. Thus the number of tested bacterial and fungal species able to produce VOCs against other microorganisms, especially those which cause plant diseases, have to be expanded through sample collections in diversified environments and soil types.

IV. Conclusion

The present investigation revealed that fungal and bacterial bio-control agents are found to be potential bioagents for the integration in the Integrated Disease Management approaches to combat Foot rot disease of areacnut. Further, the Fungi and bacteria are capable of producing a wide variety of biochemical compounds (VOCs) and are found to be more effective in inhibiting test pathogen than dual culture technique. Hence, there is need to identify the various VOCs produced by microbes which are having antimicrobial properties for the control of plant diseases. The efficacy of these compounds are to be tested under *in vitro* and glass house conditions and field trials by using either bacteria or fungal bioagents by themselves or in combination with other methods such as organic amendments or in integrated disease management approaches for eco-friendly plant diseases management.

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Table I. List of Fungal and bacterial Bio control agents used for evaluation against *Ganoderma* isolates of coconut and arecanut *in vitro*

Sl. No.	Antagonist	Strain	Source
1	*Trichoderma viride*	Tv-GKVK1	GKVK, Hebbal, Bengaluru
2	*T. viride*	Tv -AMB	
3	*T. viride*	Tv - NBAIR,	NBAIR, Hebbal, Bengaluru.
4	*T. viride*	Tv-13	IIHR, Hessaraghatta, Bengaluru
5	*T. viride*	Tv-16	IIHR, Hessaraghatta, Bengaluru
6	*T . viride*	Tv-GKVK2	GKVK, Hebbal, Bengaluru
7	*T. harzianum*	Th-58	IIHR, Hessaraghatta, Bengaluru.
8	*T. harzianum*	Th - NBAIR,	NBAIR, Hebbal, Bengaluru.
9	*T. harzianum*	Th -S	GKVK, Bengaluru
10	*T. koningi*	Tk-1	GKVK, Bengaluru
11	*Bacillus subtilis*	Bs-P42	GKVK, Bengaluru
12	*B. subtilis*	Bs-1	GKVK, Bengaluru
13	*B. subtilis*	Bs-P22	GKVK, Bengaluru
14	*Pseudomonas fluorescens*	Pf-4	GKVK, Bengaluru
15	*P.putida*	Pp-1	GKVK, Bengaluru
16	*B. amyloliquefaciens*	Ba-A6	GKVK, Bengaluru
17	*B. amyloliquefaciens*	Ba-43	GKVK, Bengaluru
18	*Actinomycetes*	Am-1	GKVK, Bengaluru

Table II. *In vitro* efficacy of bio control agents on *Ganoderma* isolates of arecanut (AG_7)

Sl. No.	Biocontrol agents	Per cent inhibition over control*	
		Dual Culture	Volatile Metabolites
1	*T. viride* (Tv-GKVK1)	34.27 (35.82)	67.41 (55.22)
2	*T. viride* (Tv -AMB)	46.89 (43.22)	68.53 (55.96)
3	*T. viride* (Tv - NBAIR)	35.78 (36.72)	71.07 (57.50)
4	*T. viride* (Tv-13)	45.64 (42.48)	57.42 (49.26)
5	*T. viride* (Tv-16)	31.19 (33.94)	57.42 (49.26)
6	*T . viride*(Tv-GKVK2)	55.78 (48.32)	53.70 (47.12)
7	*T. harzianum* (Th-58)	46.30 (42.87)	53.70 (47.12)
8	*T. harzianum*(Th - NBAIR)	35.78 (36.74)	68.53 (55.89)
9	*T. harzianum* (Th -S)	41.67 (40.18)	48.15 (43.94)
10	*T. koningi* (Tk-1)	59.86 (50.70)	63.71 (52.96)
11	*B. subtilis* (Bs-P42)	47.97 (43.84)	70.00 (56.81)
12	*B. subtilis* (Bs-1)	41.56 (40.13)	49.89 (44.95)
13	*B. subtilis* (Bs-P22)	59.27 (50.34)	70.53 (57.13)
14	*P. fluorescens* (Pf-4)	48.74 (44.27)	6.62 (14.02)
15	*P. putida* (Pp-1)	55.56 (48.19)	67.07 (54.99)
16	*B. amyloliquefaciens* (Ba-A6)	50.01 (45.02)	50.63 (45.36)
17	*B. amyloliquefaciens* (Ba-43)	63.93 (53.09)	46.59 (43.05)
18	*Actinomycetes* (Am1)	57.42 (49.26)	23.19 (28.78)
SEm ±		**5.406**	**6.670**
CD (p=0.01)		**5.177**	**5.750**
CV (%)		**5.320**	**5.417**

Note: Figure in parenthesis are arc sine transformed values
*Mean of three replications.

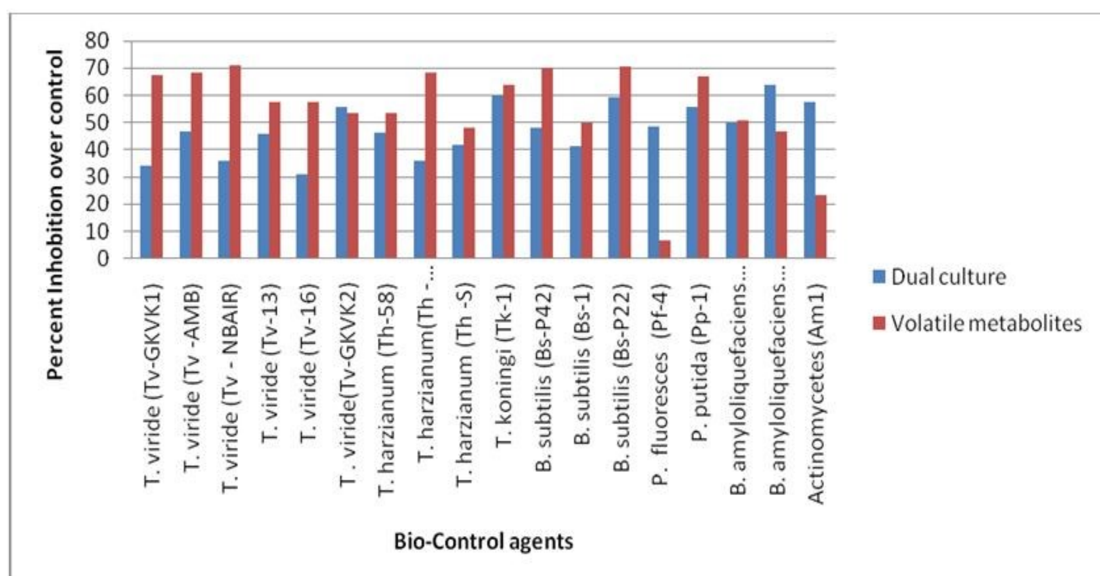

Fig. 1. *In vitro* efficacy of bio control agents on *Ganoderma* isolates (AG_7) of arecanut.

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Fungal BCAs in dual culture

Bacterial BCAs in dual culture

Volatile metabolites of Fungal BCAs

Volatile metabolites of Bacterial BCAs

Plate 1. *In vitro* efficacy of biocontrol agents on *Ganoderma* isolates (AG_7) of arecanut

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