**Ganoderma Association with the Mortality of Acacia auriculiformis**, Susceptibility to Different Hosts and Its Controls

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**Abstract**

An investigation was conducted to find out the association of *Ganoderma* spp. with the mortality of *Acacia auriculiformis* at Jahangirnagar University Campus, Bangladesh. Diseases severity of the tree was calculated where the highest (52.2 %) incidence was recorded in site-1 of the study area. Isolation and identification of the *Ganoderma* spp. and *Trichoderma* spp. were carried out. A total of 11 hosts were examined for examine the susceptibility of *Ganoderma* on host. All of the wild *Ganoderma* spp. viz., *G. lucidum*-1, *G. lucidum*-2, *G. lucidum*-3 and *G. applanatum* preferred to use saw dust of *Mangifera indica* followed by *Ceriops decandra* whereas the least performance recorded in *Albizia procera* and *Dipterocarpus turbinatus*. *Trichoderma* isolates found effective to control *Ganoderma* infestation under field conditions.

**Keywords:** *Ganoderma; Acacia auriculiformis; Trichoderma; Disease severity; Biological control*

**Introduction**

The genus *Ganoderma* includes several wood decaying fungi on living trees as well as dead trunks and stumps, and has been recorded mostly in tropical and temperate countries. Generally, *Ganoderma* spp. cause extensive heart rots of standing trees by growing in the central, non-living woody tissues. Several studies have been carried out on *Ganoderma* diseases focusing on economic damage, severity of the disease and host range in many regions such as America, Asia, the Middle East and Europe [1]. *Ganoderma lucidum* has been reported as the causal organism of the heart rot disease of 91 hosts species *Quercus* spp. [2], *Cocos nucifera* [3], *Camellia sinensis* [4], *Prunus persica* [5], *Vitis vinifera* [6], *Delonix regia* and *Cassia fistula*. According to previous studies, several *G. lucidum* strains have been identified in the *G. lucidum* complex [7] having different host specificity. Control of root rot diseases is difficult as the pathogens survive on woody material in the soil. Green mould disease caused by *Trichoderma* spp. one of the serious problem of oyster mushroom and white button mushroom. It causes large economic losses to the mushroom growers. This was agreed by [8-11]. But this *Trichoderma* spp. has ability to control various plant diseases. This study was undertaken to examine the spread of root and stem rot disease in a particular study area, identify the causal agent of the disease and control the disease by means of biocontrol agent, *Trichoderma*. So, we can use this spent mushroom compost as a biofertilizer.

**Materials and Methods**

A study was carried out to calculate disease prevalence of *Acacia auriculiformis* at four selected different sites of Jahangirnagar University Campus, Bangladesh. *Acacia auriculiformis* are the dominant trees in every site of the campus. A total of fifty trees were randomly selected in each study area. *Ganoderma* spp. viz. *G. lucidum*-1, *G. lucidum*-2, and *G. lucidum*-3 were collected from fully dead plants where as *G. applanatum* from partially dead trees. Symptomatology of infected trees due to *Ganoderma* was studied carefully.

**Identification of *Ganoderma* spp. and *Trichoderma* spp.**

In the present study, *Ganoderma* spp. was classified according to Corner [12] and Steyaert [13]. All of mycelium of four *Ganoderma* was hyaline, hyphae asceptate, basidiospores were thick walled, bitunicate, golden brown in color and ovate in shape. Colors of the colony of all wild *Ganoderma* were white (Figure 1) The morphological, microscopic and cultural characteristic features of *Ganoderma lucidum* found more or less similar with previous researchers [1,14]. There was no chlamydospore found during present study as described by previous workers.

**Disease severity index**

The trees were scored for disease classes on a scale of 0 to 4 (Table 1). The Disease Severity Index (DSI) was calculated using a modified method of Abdullah et al. [15] and Ilias [16].

**Isolation of wild *Ganoderma* spp.**

Both the pathogens and infested wood chips were cultured on PDA medium. Pieces (1 cm × 1 cm) of pileus and wood chips (1 cm × 1 cm) were placed at the center of the plate separately. Three replications for each isolate were maintained and incubated at 32 ± 2°C. All of the isolates were pure cultured on PDA plates and stored at 4°C until further use.

**Morphological and cultural characteristics**

Morphological Characterization of *Ganoderma* such as shape, size, thickness, margin, color, texture of pileus was examined. Microscopic studies were done by stereoscopic binocular microscope (OLYMPUS SZ 61, magnification 40X with Camera DP20, Japan). Hyphal features, colony characteristics were recorded.

**Isolation of antagonist**

A total of four species of *Trichoderma* i.e., *Trichoderma harzianum*,...
Four treatments combinations were used to assess susceptibility of Ganoderma. After cooling of saw dusts, fungal block each of 8 mm in size was inoculated into the test tubes containing sawdust under aseptic condition and cotton plugged. Test tubes were incubated at 32 ± 2°C temperature. Radial growth of Ganoderma spp. on the test tube was measured at 10 days intervals and was analyzed statistically by MSTAT-C program.

Treatment $T_1$ (Control) comprised of 70% Sawdust+65% moisture+inoculums (Ganoderma-1,2,3,4 separately); treatment $T_2$ of 70% Sawdust+65% moisture+0.5% CaCO$_3$ +inoculums (Ganoderma-1,2,3,4 separately); thus Treatment $T_1$ and $T_2$ made up of 70% Sawdust+65% moisture+ 30% wheat bran+2% sucrose+0.5% CaCO$_3$ +inoculums (Ganoderma-1,2,3,4 separately) and 70% Sawdust+65% moisture+ 30% rice bran+2% sucrose+0.5% CaCO$_3$ +inoculums (Ganoderma-1,2,3,4 separately).  

Field trial

A field experiments were also conducted at Jahangirnagar University Campus during April to August 2011. A total of eight treatment combinations were used in field experiments (Table 2). A total of fifty non infected plants were randomly selected in these purpose to inoculate the isolated Ganoderma to examine the capacity to infest on living host and the control of diseases prevalence using antagonists.

Mass culture of four Ganoderma isolates

The bamboo chips (3 cm) were sun dried for 10 days after cutting. Then these chips were mixed with wheat bran (30%), rice bean (30%) and 2% sugar solution with maintained 65% moisture. Then, these substrates were put into broad mouth test tubes (20 cm) and plugged with cotton. These test tubes were autoclaved for two times and waited until cooled. Test tubes were inoculated with each of four Ganoderma species. Data was collected until test tubes were filled with Ganoderma mycelium.

Preparation of Trichoderma spore suspension

At first, 20 ml of double distilled water was poured in each Petri dish having Trichoderma isolates. Then, each plate was scrapped to separate out mycelium of Trichoderma by using inoculating needle as mycelium of antagonist separated out. Then this solution was taken in plastic pot and covered with sterilized aluminum foil.

The stump or trunk region of Acacia sp. plants were holed by hammer and auger. Bamboo chips were inoculated into the plant by

### Table 1: Parameters used to calculate disease severity in the study area.

| Disease Classes | Range    | Severity of infection |
|-----------------|----------|-----------------------|
| 0               |          | Healthy plants        |
| 1               | 25-50    | Appearance of Ganoderma on the tree trunk but no remarkable damage observed in plants. |
| 2               | 50-75    | Partially top dying   |
| 3               | >75      | Fully top dying of plants and plants dried. |

Formula used to calculate disease severity index (DSI) % = Σ (A × B) × 100/ ΣB × 4 where:

A – Disease classes (0, 1, 2, 3, 4 and so on)
B – Number of plants showing that disease classes per treatment

### Table 2: Treatments used to assess the pathogenicity of Ganoderma spp. and its control using antagonistic potentiality of Trichoderma spp. at field condition.

| Treatments no. | Description                                      |
|----------------|---------------------------------------------------|
| Control        | No inoculation of Ganoderma                       |
| $T_1$          | Inoculation of G-1                                |
| $T_2$          | Inoculation of G-2                                |
| $T_3$          | Inoculation of G-3                                |
| $T_4$          | Inoculation of G-4                                |
| $T_5$          | Inoculation of G-1+addition of T. harzianum       |
| $T_6$          | Inoculation of G-2+addition of T. koningii        |
| $T_7$          | Inoculation of G-3+addition of T. viride (green strain ) |
| $T_8$          | Inoculation of G-3+addition of T. viride (yellow strain ) |

Here, G1-Ganoderma lucidum-1, G2-Ganoderma lucidum-2, G3-Ganoderma lucidum-3, G4-Ganoderma applanatum.

- 5 replication in each treatment except control where 10 replications were used.
hammer and covered with adhesive tape. The mycelial run rate per bamboo chip was observed.

**Result and Discussion**

**Symptomatological study**

*Ganoderma* infected plants showed symptoms with initially a bleached zone appeared in the wood which results delignification, drying of apical meristem or top dying of plants was the common symptoms on plants, wilting of plants, stem blackening, defoliation, white rot and root rot, loss of stiffness and finally, death of tree plants. All of the *Ganoderma* infected plants consisted at least 5 to 7 fruit bodies except *Ganoderma applanatum* which was thought to be due to delignification; caused by Dysfunctional xylem associated with large wounds on the roots which during study are in conformity with some researchers [14,19,20]. One fruit body and plant was defoliated. The symptoms recorded where infected plants contained fruit bodies except *Ganoderma applanatum*

All of the *Ganoderma* infected plants consisted at least 5 to 7 fruit bodies except *Ganoderma applanatum* which was thought to be due to delignification; caused by Dysfunctional xylem associated with large wounds on the roots which during study are in conformity with some researchers [14,19,20].

**Calculation of Disease Severity Index (DSI)**

The DSI value of *Ganoderma* at four selected sites of Jahangirnagar University Campus revealed that the highest disease incidence was found in site-1 (55.2%) in *Acacia auriculiformis* followed by site-2 (47.5%), site-4 (45%) and site-3 (36%) (Table 3). Such findings partially supported by Nur and Abdullah [22] who cited the highest DSI (70.0%) followed by site-2 found in site-1 (55.2%) in University Campus.

**Assessment of wild *Ganoderma* (four) susceptibility to different host range**

*Ganoderma lucidum-1* was susceptible *Acacia auriculiformis* due to treatment T₄, which is statistically identical to T₁, followed by T₃ at 10 days (Table 4) but treatment T₁ exhibited more susceptible at 30 days. More or less similar results found in case of *Ganoderma lucidum-2* and *Ganoderma lucidum-3*. *Ganoderma applanatum* showed no significant differences at 10 days but at 20 and 30 days only saw dusts showed more susceptible. In case of *Artocarpus chaplasha*, *Ganoderma lucidum-1* was susceptible to treatment T₄ at 10, 20, 30 days respectively (Table 4). More or less similar pattern recorded in case of *Delonix regia* (Table 5). *Ganoderma applanatum* showed better growth in sole saw dust of *Delonix regia*.

There was no clear pattern found in case of *Albizia lebbeck* for its growth (Table 4).

In case of *Dipterocarpus turbinatus*, *Ganoderma lucidum-1* and 2 showed better growth in the treatment T₄ during entire incubation period (Table 5). *Ganoderma applanatum* preferred to grow where CaCO₃ was added up to 20 days but treatment T₄ showed better performance at 30 days of inoculation. All of the *Ganoderma* spp. showed better growth performance in sole saw dust of *Ceriops decandra* (Table 5). In case of *Artocarpus heterophyllus*, *Ganoderma lucidum-1* and 3 showed better performance in treatment T₄ at 20 and 30 days in case of *Artocarpus chaplasha* and *Ceriops decandra*.

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### Table 3: Disease severity index (DSI) of site -1, 2, 3, 4 at Jahangirnagar University Campus.

| Disease Class | Range | Site 1 | Site 2 | Site 3 | Site 4 |
|---------------|-------|-------|-------|-------|-------|
| Disease Index | (%)   |       |       |       |       |
| 0             | 0     | 7     | 15    | 10    | 10    |
| 1             | 25    | 10    | 10    | 13    | 10    |
| 2             | 26-50 | 8     | 10    | 12    | 15    |
| 3             | 50-75 | 15    | 15    | 5     | 10    |
| 4             | >75   | 10    | 5     | 5     | 5     |
| DSI (%)       |       | 55.2  | 47.5  | 36    | 45    |

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### Table 4: Susceptibility of *Ganoderma* spp. to *Acacia auriculiformis*, *Artocarpus chaplasha*, *Delonix regia* and *Albizia lebbeck* at different days of inoculation.

| Treatments          | *Ganoderma lucidum 1* | *Ganoderma lucidum 2* | *Ganoderma lucidum 3* | *Ganoderma applanatum* |
|---------------------|-----------------------|-----------------------|-----------------------|------------------------|
|                     | 10 days (cm)          | 20 days (cm)          | 30 days (cm)          | 10 days (cm)           |
|                     | 20 days (cm)          | 30 days (cm)          | 10 days (cm)          | 20 days (cm)           |
|                     | 30 days (cm)          | 10 days (cm)          | 20 days (cm)          | 30 days (cm)           |
|                     | 10 days (cm)          | 20 days (cm)          | 30 days (cm)          | 10 days (cm)           |
|                     | 20 days (cm)          | 30 days (cm)          | 10 days (cm)          | 20 days (cm)           |
|                     | 30 days (cm)          | 10 days (cm)          | 20 days (cm)          | 30 days (cm)           |
| **Acacia auriculiformis** |                       |                       |                       |                        |
| T₁ (control)        | 1.93                 | 6.0 b                 | 9.00 d                | 2.70 b                 |
| T₂                 | 2.60 a               | 7.0 a                 | 10.10 c               | 3.10 a                 |
| T₃                 | 1.87 b               | 6.20 ab               | 11.30 b               | 0.00 c                 |
| T₄                 | 2.57 a               | 6.20 ab               | 13.30 a               | 2.70 b                 |
| CV (%)              | 14.94                | 7.39                 | 9.36                  | 5.54                   |
|                     | 5.54                 | 2.07                 | 1.54                  | 6.51                   |
| **Artocarpus chaplasha** |                       |                       |                       |                        |
| T₁ (control)        | 1.40 b               | 2.80 b                | 4.20 b                | 0.00 c                 |
| T₂                 | 1.67b                | 2.37 c                | 3.57 c                | 1.60 b                 |
| T₃                 | 1.40 b               | 2.80 b                | 4.20 b                | 3.00 a                 |
| T₄                 | 2.20 a               | 4.40 a                | 6.60 a                | 3.00 a                 |
| CV (%)              | 7.72                 | 3.85                 | 2.56                  | 5.00                   |
|                     | 5.00                 | 2.58                 | 1.66                  | 4.00                   |
| **Delonix regia**   |                       |                       |                       |                        |
| T₁ (control)        | 1.87 b               | 5.87 c                | 8.87 d                | 2.60 b                 |
| T₂                 | 2.60 a               | 8.70 b                | 10.27 c               | 2.97 a                 |
| T₃                 | 2.60 a               | 6.10 b                | 11.20 a               | 0.00 c                 |
| T₄                 | 2.56 a               | 6.10 b                | 13.20 a               | 2.60 b                 |
| CV (%)              | 3.66                 | 1.34                 | 3.05                  | 3.62                   |
|                     | 1.34                 | 0.94                 | 1.28                  | 5.48                   |
| **Albizia lebbeck** |                       |                       |                       |                        |
| T₁ (control)        | 1.80 b               | 5.60 c                | 8.90 d                | 1.87 d                 |
| T₂                 | 2.50 a               | 6.70 a                | 9.77 c                | 3.06 a                 |
| T₃                 | 1.87 b               | 4.77 d                | 10.07 b               | 2.97 a                 |
| T₄                 | 2.40 a               | 5.80 b                | 13.10 a               | 2.10 c                 |
| CV (%)              | 3.57                 | 1.34                 | 0.80                  | 3.59                   |

Means in a column followed by the same letter do not differ significantly at 5% level of significance (DMRT).

Treatment 1(Control) = only saw dust + inocula (*Ganoderma*-1,2,3,4 separately), Treatment 2 (T₁) = 70% Sawdust+65% moisture+ 0.5% CaCO₃ + inocula (*Ganoderma*-1,2,3,4 separately), Treatment 3 (T₂) = 70% Sawdust+65% moisture+ 30% wheat bran+2% sucrose+0.5% CaCO₃ + inocula (*Ganoderma*-1,2,3,4 separately), Treatment 4 (T₄) = 70% Sawdust+65% moisture+ 30% rice bran+2% sucrose+0.5% CaCO₃ + inocula (*Ganoderma*-1,2,3,4 separately).
preferred to use best performance in treatment T3 & T4 during entire period of study (Table 5).

-1, 2, 3 showed Ganoderma lucidum at 20, 30 days respectively (Table 5).

-2 showed better growth in treatment T3 during Ganoderma applanatum 10, 20, 30 days respectively. Whereas, -2 and -3 were susceptible to Ganoderma lucidum -3 at 20 days.

Means in a column followed by the same letter do not differ significantly at 5% level of significance (DMRT).

| Treatments                  | 10 days (cm) | 20 days (cm) | 30 days (cm) | 10 days (cm) | 20 days (cm) | 30 days (cm) | 10 days (cm) | 20 days (cm) | 30 days (cm) | 10 days (cm) | 20 days (cm) | 30 days (cm) |
|-----------------------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| T<sub>1</sub> (control)     | 1.20 c       | 2.70 c       | 4.10 b       | 1.30 b       | 3.27 b       | 4.67 b       | 1.30 b       | 3.27 b       | 4.67 b       | 1.30 b       | 3.27 b       | 4.67 b       |
| T<sub>1</sub>               | 2.70 c       | 4.70 a       | 8.50 c       | 2.30 a       | 4.50 c       | 8.70 c       | 2.30 a       | 4.50 c       | 8.70 c       | 2.30 a       | 4.50 c       | 8.70 c       |
| T<sub>2</sub> (control)     | 1.30 a       | 3.80 a       | 6.50 a       | 1.77 a       | 4.10 a       | 7.60 a       | 0.00 d       | 0.00 d       | 0.00 d       | 0.00 d       | 0.00 d       | 0.00 d       |
| T<sub>2</sub>               | 2.50 a       | 5.00 a       | 10.00 a      | 3.20 a       | 6.40 a       | 9.80 a       | 1.70 b       | 3.40 b       | 6.90 a       |
| CV (%)                      | 9.87         | 5.05         | 3.36         | 5.35         | 3.85         | 2.46         | 9.34         | 3.34         | 2.21         | 7.37         | 3.03         | 2.21         |

**Table 5:** Susceptibility of Ganoderma spp. to Diplocarpos turbinatus, Ceriops decandra, Artocarpus heterophyllus and Mangifera indica at different days of inoculation.

**Table 6:** Susceptibility of Ganoderma spp. to mixed saw dust, Tectona grandis, and Albizia procera at different days of inoculation.

In case of mixed saw dust, Ganoderma lucidum-1 was susceptible to treatment T<sub>1</sub> showed significant difference at 10 days but G. lucidum-1, 2 showed better performances in treatment T<sub>2</sub> at 30 days. G. lucidum-3 and Ganoderma applanatum were prone to sole saw dust at 10, 20, 30 days respectively upto entire study (Table 6) G. lucidum-1 and 2 were commonly susceptible to Tectona grandis due to the treatment T<sub>2</sub> at 10, 20, 30 days respectively. Whereas, Ganoderma lucidum-2 and Ganoderma applanatum showed better growth in treatment T<sub>3</sub>, during entire period of study (Table 5). Ganoderma lucidum-1, 2, 3 showed better performances to all of the treatments at 30 days. Ganoderma applanatum preferred to use best performance in treatment T<sub>2</sub> at 30, 30 days respectively (Table 5).
10 days and treatment T4 at 20 days. But at 30 days G. lucidum-1 and 2 showed better growth in treatment T1 and T2 respectively. G. lucidum-3 and G. applanatum showed more growth performance in treatment T4 at 30 days (Table 6). In case of G. lucidum-1, 2, 3 showed better performances in the treatment T2 at 30 days whereas G. applanatum showed no run rate in any treatments at any days (Table 6).

All of the Ganoderma spp., i.e., G. lucidum-1, G. lucidum-2, G. lucidum-3 and G. applanatum preferred to use saw dust of Mangifera indica followed by Ceriops decandra. This might be due to the presence of readily usable materials for its growth. In most of the cases, the least performance showed in Albizia procera and Dipterocarpus turbinatus might be the presence of secondary metabolites such as tannins, resins, and gums in wood. Root diseases caused by Ganoderma spp. seriously affected growth of Acacia spp. [24], Acacia auriculiformis and Acacia nilotica in India and Pakistan [25], Red rot disease of Acacia mangium caused by Ganoderma sp. reported in Malaysia [26]. Acacia auriculiformis affected by root rot disease caused by Ganoderma and Phellinus spp. in Papua New Guinea [27], heart rot disease caused by Ganoderma lucidum recorded in Quercus spp., Cocos nucifera, Camelia sinensis, Prunus persica, Vitis vinifera, Cassia nodosa, Cassia fistula, Delonix regia, others 144 hosts in India [1].

Pathogenicity of Ganoderma spp. and antagonistic potentiality of Trichoderma spp.

In vivo pathogenecity of four Ganoderma spp. showed colonization of mycelium within 5 months, that means trees inoculated with bamboo chips of Ganoderma lucidum-1, G. lucidum-2, G. lucidum-3, G. applanatum showed mycelial run rate in the surroundings of inoculated area. In case of Ganoderma applanatum, mycelial run rate was just initiated (Figure 2). In vivo evaluation of potentiality of Trichoderma against Ganoderma spp. also studied. Bamboo chips of four Ganoderma spp. were inoculated with four selected Trichoderma spp. showed no mycelial run rate of Ganoderma spp., even Trichoderma spp. was sporulated over the inoculated area. Ganoderma applanatum as the most degradative wood colonizer [14]. The pathogenicity of Ganoderma spp. and the inhibitory effect of Trichoderma on Ganoderma spp. was followed earlier research [28].

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Figure 2: Photographs showing in vivo potentiality of Trichoderma and pathogenecity of Ganoderma spp.

a. Sporulation of Trichoderma over Ganoderma inoculated area
b. Colonization of Ganoderma mycelia
c. Colonization of Ganoderma lucidum-1 mycelia on the inoculated area
d. Colonization of Ganoderma lucidum-2 mycelia on the inoculated area
e. Colonization of Ganoderma lucidum-3 mycelia on the inoculated area
f. Colonization of Ganoderma applanatum mycelia on the inoculated area (just initiated).
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