# "Fungicidal Effect of Various Extracts of *Chlorophytum borivilianum* (Sant. F.) on Different *Aspergillus* Species"

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## Abstract

*Chlorophytum borivilianum* of Liliaceae is widely distributed in India. This plant is mostly used to treat oligospermia, pre and post natal symptoms, arthritis, diabetes and dysuria. It is also antiviral, anticancer, immunomodulatory, antidiabetic, antistress, aphrodisiac, antimicrobial and anti-inflammatory in nature. It is even used as a vitalizer and nutritic health tonic. *Aspergillus* species are known to cause seasonal inter abdominal, pulmonary, lymph node and cutaneous infections. Some of the *Aspergillus* species produce toxins, attack growing crops and colonise in economically important fruits, seeds and other plant parts, thereby affecting plants and animals including human beings. They also cause rot diseases in fruits and spoil bread, jam, jely, bars and other economically important products. *Aspergillus flavus* and *A. parasiticus* produce mycotoxins, which are detrimental to human beings and reported to be carcinogenic, hepatotoxic, teratogenic and immune suppressive. Ethanol, methanol and distilled water hot extracts of leaves were investigated for their antifungal activity. The growth inhibition was determined using food poisoning method against different *Aspergillus* species. Over all, methanolic leaf extract showed more antifungal activity than ethanolic and distilled water leaf extracts. *A. niger* was more sensitive followed by *A. parasiticus*-456, whereas *A. flavus* was resistant to methanolic extract. *A. parasiticus*-456 was more sensitive to ethaonolic extract followed by *A. flavus* and *A. oryzae*. *A. oryzae* and *A. niger* were resistant, whereas all other selected *Aspergillus* species were sensitive to distilled water extract. Standard antifungal agent was more inhibitory than all other extracts tried.

**KEYWORDS**: *Chlorophytum borivilianum* (Sant. F.), antifungal activity, *Aspergillus* species and Safed Musali.

## INTRODUCTION:

The extensive use of fungicides to control plant pathogenic fungi has become crucial in modern Agriculture. Non judicious use of these compounds has led to emergence of new strains of pathogens, which are resistant to the available commercial pesticides. Pesticides also pollute environment including soil (Agnihotri *et. al*., 1999) and are not economically feasible to common farmers. Many fungicides have side effects e.g. Miconazole causes purities, anemia and thrombocytosis (Anke, 1997). Many of these pesticides ($PCN_{13}$, BHC, DDT and Aldrin) are banned due to their adverse side effects. Chemical fungicides enter in water and food chain and remain in tissues. They severely affect human beings, plants and other animals.

Therefore, it necessary to evolve an alternate coherent pest management through the use of botanical pesticides. Botanical pesticides can be effectively used to control growth of plant and animal pathogenic fungi, thereby inhibiting the aflatoxin production by *Aspergillus* species (Hutson and Junshi, 1998). *Aspergillus* species are known to cause seasonal inter abdominal, pulmonary, lymph node and cutaneous infections. Some of the *Aspergillus* species produce toxins, attack growing crops and colonise in economically important fruits, seeds and other plant parts, thereby affecting plants and animals including human beings. They also cause rot diseases in fruits and spoil bread, jam, jely, bars and other economically important products. *Aspergillus flavus* and *A. parasiticus* produce mycotoxins, which are detrimental to human beings and are reported to be carcinogenic, hepatotoxic, teratogenic and immune suppressive. *Aspergillus* is recognized as a common fungal infection in immunocompromised patients (Beuchat, 1987). They are also allergens (Moghtader, 2003). Day by day, there is increasing trend towards use of green or botanical pesticides due to their safety and efficacy.

*Chlorophytum borivilianum* (Sant. F.) locally known as Safed Musali, belongs to family of Liliaceae. It is native to tropical and subtropical regions of Africa and Asia and found in Gujarat, Madhya Pradesh and Central Decan Plateau. Its tuber are used in Aurvedic medicines as it contains alkaloids and natural steroid saponins. This plant is a small perennial herb (Naik, 1998).

This plant is mostly used to treat oligospermia, pre and post natal symptoms, arthritis, diabetes and dysuria. It is also antiviral, anticancer, immunomodulatory, antidiabetic, antistress, aphrodisiac, antimicrobial and anti-inflammatory in nature. It is even used as a vitalizer and nutritic health tonic (Deore and Khadabadi, 2010).

By considering the above importance, *Chlorophytum borivilianum* was selected to study its antifungal activity against different *Aspergillus* species.

## MATERIALS AND METHODS:

### Plant material:

The plants of *Chlorophytum borivilianum* (Sant. F.) were collected from Barad village of Nanded district in the state of Maharashtra, India. It was authenticated with help of regional flora. The roots were cleaned, dried and powdered. The powder was sieved and stored in airtight container. This powder was used for making different extracts.

### Extraction of plant material:

Powdered plant material was extracted using soxhlet extractor for 6 to 8 hrs. with three different solvents viz. ethyl alcohol, methyl alcohol and distilled water. 20 gm of plant material was separately extracted in 300 ml of each solvent. The extracts were then filtered through Whatman filter paper No. 1. Solvents were evaporated under reduced pressure to yield residue and was dissolved in DMF. This extract was used for testing antifungal activity.

### Food poisoning method:

Three extracts were screened for antifungal activity by food poisoning method against

different *Aspergillus* species viz. *Aspergillus flavus* and *A. parasiticus,* which are producers of aflatoxin and *A. nigar and A. oryzae* (Hutson and Janshi, 1998; David *et. al*., 1998 and Bruce *et. al*., 2009).

In this technique, radial growth of different fungi inhibited by the extract was studied on potato dextrose agar plates. Growth inhibition was studied after different intervals of 24, 48 and 72 hrs. The concentrations of extracts were used 600, 800 and 1000 µg/ml. The plates were drilled with the help of sterilized cork borer (0.8 cm size) and to this activated 24 hrs. growth of above fungi was added and radial growth was measured at different time intervals. Then percentage of inhibition was calculated using following formula (Karapinar, 1990).

Percentage of inhibition (I) = C – T / C x 100.

Where

C = Radial growth in control – disc diameter (cm)

T = Radial growth in treated – disc diameter (cm)

I = Inhibition percentage.

**RESULTS AND DISCUSSION:**

**Effect of methanolic root extract of *C. borivilianum* on different *Aspergillus* species:**

The methanolic root extract of *C. borivilianum* showed considerable antifungal activity. The highest percentage of inhibition was shown against *A. niger* (83) followed by *A. parasiticus - 456* (81.0), *A. parasiticus* (36.6) and *A. oryzae* (33.3), where as lowest percentage of inhibition was shown against *A. flavus* (21.2), indicating that *A. niger* was most sensitive, whereas *A. flavus* was most resistant amongst all selected pathogenic fungi.

As the concentration of extract increases, there is increase in percentage of inhibition for *A. niger, A. flavus* and *A. parasiticus - 456*. However, increase in concentration of methanolic root extract decreases the percentage of inhibition in *A. parasiticus* and *A. oryzae.* As the incubation period increases, the percentage of inhibition of all selected fungi also increases. The results are summarized in table No. 1.

Methanolic extract showed antifungal activity against all selected pathogenic fungi. Such activity is also reported (Ambasta, 1992; Chopra *et. al*., 1996 and Gomathi and Kannabiran, 2000). It contrast to this, increase in concentration of extract increased the percentage of inhibition.

**Effect of Ethanolic root extract of *C. borivilianum* on different *Aspergillus* species:**

The ethanolic root extract of *C. borivilianum* also showed considerable antifungal activity. The highest percentage of inhibition was shown against *A. parasiticus* - 456 (81.8) followed by *A. flavus* (75.0), *A. oryzae* (65.2) and *A. niger* (64.5). The lowest percentage of inhibition was shown against *A. parasiticus* (41.6) indicating that *A.*

*parasiticus - 456* was most sensitive, whereas *A. parasiticus* was most resistant amongst all selected pathogenic fungi.

As the concentration of extract increases, the percentage of inhibition decreases. The time of incubation period is inversely proportional to inhibition percentage. Overall, ethanolic root extract was less inhibiting than methanolic root extract. The results are summarized in table No. 2.

Ethanolic extract showed antifungal activity against selected pathogenic fungi like that of earlier reported (Sharma *et. al.*, 2002; Kagne *et. al.*, 2012 and Balkhande and Surwase, 2013).

**Effect of distilled water root extract of *C. borivilianum* on different *Aspergillus* species:**

The aqueous root extract of *C. borivilianum* showed moderate antifungal activity. *A. parasiticus-456* was most sensitive showing highest percentage of inhibition (81.8), followed by *A. flavus* (58.8), *A. parasiticus* (36.3), *A. niger* (14.8) and *A. oryzae* (14.0).

In aqueous root extract also, as the percentage of extract increases, the percentage of inhibition decreases. As the incubation period increases, the percentage of inhibition increases up to 48 hrs of incubation only. The results are summarized in table No. 3.

Distilled water extract showed antifungal activity against all selected pathogenic fungi like that of earlier reported (Chandrasekaran and Venkatesalu, 2004).

This support the investigation that the antifungal activity may be due to the presence of chemical compounds and oil (Alexopoulus *et. al.*, 2003; Allcroft *et. al.*, 1968 and Chandniwala, 1998).

So in comparison methanolic extract has more antifungal activity and standard antibiotic is more inhibitory in nature.

Hence from the above results, the roots of *C. borivilianum* can be used for Integrated Pest Management (IPM) to control growth of all pathogenic fungi and can also be utilized to develop antifungal agents.

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**Table No. 1: Effect of methanolic root extract of *C. borivilianum* on different *Aspergillus* species:**

| Sr. No. | Name of fungus | Extract/ Antibiotic | Concentration (ug/ml) | Radial growth in cm* | | |
|---|---|---|---|---|---|---|
| | | | | 24 Hrs. | 48 Hrs. | 72 Hrs. |
| 1. | *Aspergillus parasiticus* | Extract | 600 | 1.8 (16.6) | 2.9 (04.5) | 3.9 (06.0) |
| | | | 800 | 1.9 (26.6) | 2.7 (36.6) | 3.8 (31.8) |
| | | | 1000 | 1.8 (09.0) | 2.8 (04.7) | 3.8 (03.2) |
| | | Antibiotic (Fluconazole) | 10 | 1.7 (59.0) | 3.3 (40.4) | 5.0 (10.6) |
| | | | 20 | 1.6 (65.2) | 3.0 (35.2) | 4.5 (13.9) |
| 2. | *Aspergillus parasiticus -456* | Extract | 600 | 1.6 (42.8) | 1.9 (57.6) | 3.1 (43.9) |
| | | | 800 | 1.9 (15.3) | 1.9 (54.0) | 3.5 (25.0) |
| | | | 1000 | 1.8 (81.0) | 3.0 (60.7) | 4.1 (44.7) |
| | | Antibiotic (Fluconazole) | 10 | 1.9 (52.1) | 3.4 (40.9) | 5.1 (15.6) |
| | | | 20 | 1.5 (68.1) | 2.2 (37.1) | 4.7 (18.7) |
| 3. | *Aspergillus flavus* | Extract | 600 | 1.7 (18.1) | 2.5 (19.0) | 3.4 (13.3) |
| | | | 800 | 1.6 (20.1) | 2.4 (05.8) | 3.2 (04.0) |
| | | | 1000 | 1.6 (11.1) | 2.2 (17.8) | 2.4 (21.2) |
| | | Antibiotic (Fluconazole) | 10 | 4.4 (14.2) | 4.5 (15.9) | 4.8 (23.0) |
| | | | 20 | 3.9 (11.4) | 4.0 (31.9) | 4.4 (12.0) |
| 4. | *Aspergillus niger* | Extract | 600 | 2.3 (06.2) | 3.5 (03.5) | 3.6 (30.0) |
| | | | 800 | 2.1 (25.2) | 3.4 (03.7) | 3.6 (28.2) |
| | | | 1000 | 1.9 (83.0) | 3.1 (17.8) | 3.4 (29.2) |
| | | Antibiotic (Fluconazole) | 10 | 1.7 (59.0) | 2.2 (41.6) | 2.3 (50.0) |
| | | | 20 | 1.5 (74.0) | 2.0 (60.0) | 2.0 (73.6) |
| 5. | *Aspergillus oryzae* | Extract | 600 | 4.0 (33.3) | 7.8 (22.5) | 8.9 (01.2) |
| | | | 800 | 4.0 (13.5) | 7.5 (08.2) | 9.0 (01.2) |
| | | | 1000 | 3.6 (33.3) | 7.4 (18.5) | 8.9 (01.2) |
| | | Antibiotic (Fluconazole) | 10 | 4.5 (01.2) | 9.0 (14.0) | 9.0 (16.0) |
| | | | 20 | 4.6 (22.4) | 9.0 (20.0) | 9.0 (25.5) |

*Radical growth including 0.8 cm disc diameter measured after 24, 48 and 72 hrs. A value in bracket indicates percentage of inhibition.

**Table No.2: Effect of ethanolic root extract of *C. borivilianum* on different *Aspergillus* species:**

| Sr. No. | Name of fungus | Extract/ Antibiotic | Concentration (ug/ml) | Radial growth in cm* | | |
|---|---|---|---|---|---|---|
| | | | | 24 Hrs. | 48 Hrs. | 72 Hrs. |
| 1. | *Aspergillus parasiticus* | Extract | 600 | 2.0 (14.2) | 2.2 (12.5) | 2.2 (41.6) |
| | | | 800 | 2.1 (07.1) | 2.3 (11.7) | 3.1 (04.1) |
| | | | 1000 | 1.9 (35.2) | 2.2 (36.3) | 1.9 (05.6) |
| | | Antibiotic (Fluconazole) | 10 | 1.7 (59.0) | 3.3 (40.4) | 5.0 (10.6) |
| | | | 20 | 1.6 (65.2) | 3.0 (35.2) | 4.5 (13.9) |
| 2. | *Aspergillus parasiticus -456* | Extract | 600 | 1.4 (62.5) | 2.0 (42.8) | 2.4 (20.0) |
| | | | 800 | 2.1 (38.0) | 2.9 (81.8) | 2.6 (10.5) |
| | | | 1000 | 1.2 (07.7) | 1.7 (59.0) | 2.6 (25.0) |
| | | Antibiotic (Fluconazole) | 10 | 1.9 (52.1) | 3.4 (40.9) | 5.1 (15.6) |
| | | | 20 | 1.5 (68.1) | 2.2 (37.1) | 4.7 (18.7) |
| 3. | *Aspergillus flavus* | Extract | 600 | 1.5 (41.6) | 2.1 (23.5) | 2.4 (15.7) |
| | | | 800 | 1.1 (75.0) | 1.2 (58.8) | 1.7 (07.1) |
| | | | 1000 | 1.4 (25.0) | 1.6 (20.0) | 1.8 (09.1) |
| | | Antibiotic (Fluconazole) | 10 | 4.4 (14.2) | 4.5 (15.9) | 4.8 (23.0) |
| | | | 20 | 3.9 (11.4) | 4.0 (31.9) | 4.4 (12.0) |
| 4. | *Aspergillus niger* | Extract | 600 | 2.0(25.0) | 2.9 (04.5) | 3.1 (28.0) |
| | | | 800 | 2.1 (61.7) | 3.0 (45.0) | 3.1 (43.9) |
| | | | 1000 | 1.9 (64.5) | 2.9 (48.7) | 3.4 (45.2) |
| | | Antibiotic (Fluconazole) | 10 | 1.7 (59.0) | 2.2 (41.6) | 2.3 (50.0) |
| | | | 20 | 1.5 (74.0) | 2.0 (60.0) | 2.0 (63.6) |
| 5. | *Aspergillus oryzae* | Extract | 600 | 5.4 (16.3) | 5.9 (17.7) | 6.0 (22.3) |
| | | | 800 | 5.5 (20.3) | 6.0 (17.4) | 5.5 (27.6) |
| | | | 1000 | 2.9 (62.5) | 3.1 (64.0) | 3.2 (65.2) |
| | | Antibiotic (Fluconazole) | 10 | 4.5 (01.2) | 9.0 (13.0) | 9.0 (17.0) |
| | | | 20 | 4.6 (22.4) | 9.0 (18.0) | 9.0 (23.0) |

*Radical growth including 0.8 cm disc diameter measured after 24, 48 and 72 hrs. A value in bracket indicates percentage of inhibition.

**Table No. 3: Effect of distilled water root extract of *C. borivilianum* on different *Aspergillus* species:**

| Sr. No. | Name of fungus | Extract/ Antibiotic | Concentration (ug/ml) | Radial growth in cm* | | |
|---|---|---|---|---|---|---|
| | | | | 24 Hrs. | 48 Hrs. | 72 Hrs. |
| 1. | *Aspergillus parasiticus* | Extract | 600 | 1.8 (09.0) | 2.2 (12.5) | 3.9 (03.1) |
| | | | 800 | 1.8 (09.0) | 2.3 (11.7) | 3.6 (20.0) |
| | | | 1000 | 1.9 (08.3) | 2.2 (36.3) | 3.9 (36.3) |
| | | Antibiotic (Fluconazole) | 10 | 1.7 (59.0) | 3.3 (40.4) | 5.0 (10.6) |
| | | | 20 | 1.6 (65.2) | 3.0 (35.2) | 4.5 (13.9) |
| 2. | *Aspergillus parasiticus -456* | Extract | 600 | 1.9 (20.0) | 2.0 (42.8) | 4.2 (17.0) |
| | | | 800 | 1.7 (25.0) | 2.9 (81.8) | 4.0 (15.7) |
| | | | 1000 | 1.9 (08.3) | 1.7 (59.0) | 3.9 (16.2) |
| | | Antibiotic (Fluconazole) | 10 | 1.9 (52.1) | 3.4 (40.9) | 5.1 (15.6) |
| | | | 20 | 1.5 (68.1) | 2.2 (37.1) | 4.7 (18.7) |
| 3. | *Aspergillus flavus* | Extract | 600 | 1.6 (11.1) | 2.1 (23.5) | 3.5 (10.0) |
| | | | 800 | 1.6 (11.8) | 1.2 (58.8) | 3.4 (07.1) |
| | | | 1000 | 1.5 (12.5) | 1.6 (20.0) | 3.0 (24.0) |
| | | Antibiotic (Fluconazole) | 10 | 4.4 (14.2) | 4.5 (15.9) | 4.8 (23.0) |
| | | | 20 | 3.9 (11.4) | 4.0 (31.9) | 4.4 (12.0) |
| 4. | *Aspergillus niger* | Extract | 600 | 1.9 (08.3) | 2.9 (12.5) | 4.5 (02.6) |
| | | | 800 | 1.9 (08.3) | 3.1 (14.8) | 4.5 (07.5) |
| | | | 1000 | 1.9 (10.2) | 3.2 (07.6) | 4.7 (09.0) |
| | | Antibiotic (Fluconazole) | 10 | 1.7 (59.0) | 2.2 (41.6) | 2.3 (50.0) |
| | | | 20 | 1.5 (74.0) | 2.0 (60.0) | 2.0 (63.6) |
| 5. | *Aspergillus oryzae* | Extract | 600 | 5.1 (14.0) | 9.0 (12.0) | 9.0 (08.0) |
| | | | 800 | 5.5 (06.3) | 9.0 (04.0) | 9.0 (02.0) |
| | | | 1000 | 5.0 (08.6) | 9.0 (07.0) | 9.0 (04.0) |
| | | Antibiotic (Fluconazole) | 10 | 4.5 (21.2) | 9.0 (16.0) | 9.0 (16.7) |
| | | | 20 | 4.6 (22.4) | 9.0 (20.0) | 9.0 (18.0) |

*Radical growth including 0.8 cm disc diameter measured after 24, 48 and 72 hrs. Values in bracket indicate percentage of inhibition.

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