



# THE EFFECTS OF A COMBINATION OF FIVE DIFFERENT PLANT EXTRACTS OF MEDICINAL PLANTS ON THE GROWTH AND SPORULATION OF PATHOGENIC FUNGI PREVALENT ON MAKHANA PLANT

Sangeeta Kumari<sup>1</sup>, A. M. Mishra<sup>2</sup>

<sup>1</sup> Research Scholar, LNMU Darbhanga

<sup>2</sup> Associate Professor, P. G. Department of Botany, LMNU Darbhanga

## ABSTRACT

WHO, in its bulletin has advocated the use of alternate chemical ingredients for protection of crops instead of commercial chemicals in the form of fungicides, Pesticides and insecticides. Their residual on the crops and their produce results the different health hazards which has now become a challenging task for the survival of mankind. In market there are a lot of commercial chemicals available in the form of fungicides which farmer apply on their infected plants. Although these chemical are useful in controlling the spread of devastating fungi but their little amount of residual is sufficient to cause different health hazards which is the direct result of increasing mortality rate. Taking these problems in their view the World Health Organization now has advocated the search of alternate chemical in place of harmful one and it will be possible only when we may give an out look towards the natural wealth the plant. In this research work, I have considered a standard extract which will be the combination of the extract of five important. Medicinal plants including *Azadirachta indica*, *Ocimum sanctum*, *Calotropis procera*, *Chrysopogon zizanioides*, *Cascabela thevetia*. This extract when was applied on four pathogenic fungi of Makhana plant showed satisfactory result.

**KEYWORDS:** Antifungal, Plant Extract, Pathogenic Fungi, Medicinal Plants, Fungicides

## INTRODUCTION

To manage the human health, there is importance of the healthy food. Now a days it is observed that the food which are in various modes, become not free from the access of chemicals because they are found to be chemically treated. Those chemicals loaded on the food materials are directly engulfed by the human beings through their food practices and these chemicals when enter inside the human body are toxic in nature and are responsible for creating a number of Physical abnormalities in the human body like Gastric Disorders, Peptic Ulcer, Intestinal Cancer, Lung Disorder, Kidney Damage, Blood Poisoning and the Heart Failure. So, there is the need of producing the food stuffs free from the chemicals and finally producing the bold and bonafide health. Here there is question that how the food Stuff become loaded with the harmful chemicals? The answer is that the standing crops become the platform of various pathogenic agents in the form of Bacterias, Viruses and Fungi together with also the different types of Insects and Pests. These pathogenic agents are responsible for causing the serious damage of the agricultural crops and finally into the agricultural production by causing the different diseases. To get off free from the ravages of these harmful pathogenic agents, the farmer apply different types of chemicals formulated for the destruction. of these harmful pathogenic agents and are available in the market in the form of Bactericides, Nematicides, Fungicides, Insecticides and Pesticides with different trade names. No doubt, the application of these chemicals control the growth and development of the pathogenic agents which are prevalent on the agricultural crops but they are stable and persistent which persist on the agricultural produce till the formation of grains and fruits. Some portions of it may enter inside the agricultural products and stay there.

When they are harvested and sold in the market become loaded with these harmful chemicals. Now, there is need of search out the alternative of these chemicals from the herbal source. Most of the herbs contain the medicinal ingredients in the form of the Phenols, Terpenoids, Alkaloids, Flavonoids which according to Mahadevan 1982 are Prohibitins which prohibits or inhibits the growth and sporulation of the harmful pathogenic fungi prevalent on the agricultural crops. These chemicals hidden inside the herbal source become harmless for the human beings who take the treated agricultural produce but harmful for the pathogenic fungi. It has been observed that these chemicals are temporarily existing on the agricultural plant that most of its part get evaporated in the environment and the residual may be eaten by bacterial agents. Makhana (*Euryale ferox salisbury*) is an important food stuffs for human beings which is rich source of Protein and contain medicinal ingredients, Jha et.al-1991. They are generally produced in shallow and organic rich water. Naturally they are growing as healthy leaves but one of the harmful fungus known as *Alternaria* spp. cause the serious damage to the leaves by causing the harmful disease known as Leaf Blight. Due to the appearance of this disease, a large portion of photosynthetic area of the leaves get damaged resulting in the form of brown, burnt appearance. The product of Makhana usually in the form of Makhana Puff are sold in market and exported into the foreign countries. Before trading they are tested for the health certificate. If those Makhana puffs become loaded with harmful chemicals are out rightly rejected from the foreign market.

Considering the above views, I have decided to test a mixture of five medicinal plant on the health of four pathogenic fungi

causing serious diseases on Makhana plant. A brief account of these medicinal plants are as such.

### Neem (*Azadirachta indica*)

#### Brief Account of Plant:

*Azadirachta indica* commonly known as Neem, Margosa, Indian lilac, is a tree in the mahogany family Meliaceae. It is a medium-sized tree reaching 15 to 30 m in height with a large round crown 10-20 m in diameter. Neem has a deep tap root and is a mycorrhizal-dependent species.

- The leaves are alternate, Petiolated, clustered at the end of branches, unequally Pinnate, glabrous and dark glossy green at maturity, 20-40 cm in length and bearing 10-20 leaflets.
- The flowers are numerous fragrant white and borne in large clusters (up to 30 cm long).



- Neem fruits are 1-2 cm long drupes, smooth and green with white milky Juice when unripe, turning a yellow to brown when mature. They have a thin epicarp, a mucilaginous fleshy mesocarp and a hard endocarp.

They contain a variable number of ovoid (1-2 c.m.) oil seeds.

#### Chemical Constituent:

It is considered that *Azadirachta indica* shows therapeutic role due to rich source of antioxidant and other valuable active compound such as Azadirachtin (C<sub>35</sub> H<sub>44</sub> O<sub>16</sub>), Nimbolinin, Nimbin, Nimbidin, Nimbidol, Epoxyazadiradione (C<sub>28</sub> H<sub>34</sub> O<sub>6</sub>), Phytol, Gedunin, Salannin, Quercetin.

#### Medicinal Value:

Neem (*A. indica*) Plants parts shows antimicrobial role through inhibitory effect on microbial growth/ Potentiality of cell wall break down. Azadirachtin, a complex tetranortriterpenoid limonoid present in Seeds, is the key constituent responsible for both antifeedant and toxic effects in insects.

- Neem has proved effective against certain fungi as fungicides. Neem preparations showed toxicity to culture of 14 common fungi including members of the following genera like Trichophyton, Epidermophyton, Microsporium, Trichosporum, Geotrichum, Candida.
- Pharmacological activities of *Azadirachta indica* in disease management through the modulation of various activities like Antidiabetic, Neuroprotective, Antiviral Antibacterial, Antifungal, Antioxidant, Anti-inflammatory, Antimalarial, Hepatoprotective, Antinephrotoxicity, wound healing effect, Immunodulatory effect, Anticancer activity.

### Tulsi (*Ocimum sanctum*)

#### Brief Account of Plant:

Tulsi is classified as part of the Lamiaceae family, which

includes flowering plants. *Ocimum* is a genus of aromatic herb and Shrub. Lamiaceae is a family of Plants that are native to tropical and subtropical regions. *Ocimum sanctum* (Tulsi) is an erect, much branched Sub-Shrub (30-60 cm tall) with simple opposite green or purple leaves that are strongly scented and hairy stems. Leaves have petiole and are ovate, up to 5 cm long, usually somewhat toothed. Flowers are purplish in elongate racemes in close Whorls. It is cultivated for religious and medicinal purposes and for its essential oil.



#### Chemical Constituent:

*Ocimum sanctum* is an aromatic Plant. The main Chemical constituent of Tulsi are: Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, carvacrol, Linalool,  $\beta$  caryophyllene, germacrene. Volatile Oil, fixed oil also obtained from the leaves of medicinal herbs. Monoterpene are obtained from the volatile oils such as, camphene, myrcene, sabinene, in which some monoterpene produced oxygen such as linalool, borneol. Phytochemical analysis of this medicinal herb can identify the nature of compounds present in the extract of *Ocimum sanctum*. It is also for identify the bioactive compound and their effect.

#### Medicinal Value:

Tulsi is cultivated for religious and traditional medicine purposes and also for its essential oil. It is widely used as herbal tea, commonly used in Ayurveda and has a place within the Vaishnava tradition of Hinduism, in which devotees perform worship involving holy basil plants or leaves.

- Tulsi is called the wonder herb or sometimes the holy herb because of its medicinal properties. Tulsi is also used to treat Heart disease and fever. It is used to cure fever, common cold, sore throat, Kidney Stones, headaches and Asthma.
- Tulsi might be an antipyretic agent, anti-inflammatory, antiemetic (Prevent vomiting), analgesic, anti-asthmatic, diaphoretic (induces sweating), Anticancer, hepatoprotective agent, hypolipidemic activity (lower cholesterol) and antidiabetic also.
- *Ocimum sanctum* considered as a potent adaptogen. Tulsi has a unique combination of Pharmacological actions that Promote well being and resilience.

### *Chrysopogon Zizanioides* ( Khus-Khus )

#### Brief Account of *C. Zizanioides*:

*Chrysopogon zizanioides*, commonly known as vetiver and khus is a perennial bunch grass of family poaceae.



Vetivers widely cultivated in tropical regions of the world. It has thick fibrous adventitious roots. Which are aromatic and highly valued. Unlike most grasses it forms horizontally spreading mat like root systems. Khus roots plant are long, thin and fibrous and can reach up to 4 meters in depth. The stems are tall and the leaves are long, thin and rather rigid. Leaves are large and tufted with bunchy grasses. It grows upto the height of 5 feet. Inflorescence are spikelets and there are three stamens. The flowers are brownish purple. Khus plants have a gregarious habit of growing and they grow in clusters.

#### Chemical Composition:

The roots of this grass on steam distillation yield an essential oil mainly consisting of sesquiterpenes (3-4 %), sesquiterpenols (18-25 %) and sesquiterpenones (7-8 %). Among these the major economically important active compounds are khusimol, a- vetivone, b- vetivone which constitute about 35% of oil.

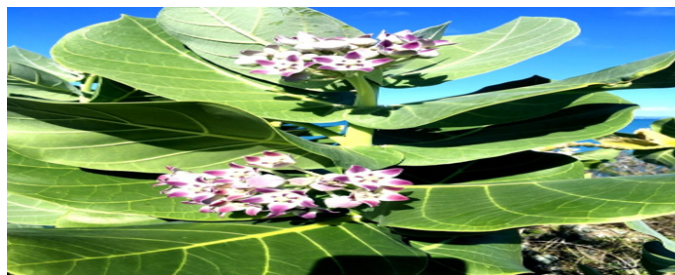
#### Medicinal Importance:

Khus-khus has been found effective in treating insomnia. The oil extracted from khus-khus relaxes and soothes the tired muscles and the mind. It help in reducing inflammation.

#### *Calotropis procera* (Arka)

##### Brief Account of Plant:

*Calotropis procera* is an evergreen perennial shrub of the family Asclepiadaceae, mainly found in arid and semi-arid region. It is a large shrub growing to 4 m ( 13 ft ) tall. The branch bears opposite leaves which are simple, petiolate, ovate with entire margin and pointed tip. The young leaves are covered by small, soft, valvate hairs and bears white milky latex. The older part of the stem is hard, smooth and covered by bark. The younger stem is green, shiny, soft and glabrous which also bears the white milky latex. Leaves with acute subsessile apices extraaxillary umbellate panicle inflorescence with purple corolla and erect lobes. It has clusters of waxy flowers that are either white or lavender in colour. Each flower consist of 5 pointed petals and a small "crown" rising from the center which holds the stamens. The aestivation found in *calotropis* is valvate.



*Calotropis* is a large bushy shrub with decussate, obovate, coriaceous, auriculate, leaves with acute subsessile apices

extraaxillary umbellate panicle inflorescence with purple corolla and erect lobes.

#### Chemical Constituent:

*Calotropis procera* contained many biological active chemical group including Cardenolides, Steroids, Tannins, Glycosides, Phenols, Terpenoids, Sugar, Flavonoids, Alkaloids and Saponins. Latex produced by *C. procera* contains many compounds like alkaloids. They are Calotropin, Catotoxin, Calcilin and Gigantean. These alkaloids are very harmful and having poisonous properties.

#### Medicinal Value :-

*Calotropis* contains chemicals that might help thin mucuous and make it easier to cough up. *Calotropis* has shown some activity against pain, inflammation, bacteria, fever and ulcers caused by alcohol and medications such as aspirin, indomethacin (indocin) and others. The leaves of *C. procera* are said to be valuable as an antidote for snake bite, sinus fistula, rheumatism, mumps, burn injuries and body pain. The leaves are also used to treat jaundice.

#### *Cascabela Thevetia* (Kaner)

##### Brief Account of Plant:

*Cascabela thevetia* is an evergreen tropical shrub belong to family Apocynaceae. It is a poisonous plant native throughout Mexico and in Central America and cultivated widely as an ornamental. It is effectively drought resistant and tolerant to high temperature, hence found in various state of India. It is commonly known as kaner. It is a relative of Nerium oleander, giving it is a common name yellow oleander.



*C. thevetia* is an evergreen shrub with thick and pointy leaves arranged in pairs or whorls of 3 along the stem. This fast growing tropical shrub produces clusters of reddish 5-petalled flowers at the end of branches and are sweetly scented. Its stem is green turning silver/grey as it ages. Its leaves are willow-like, linear-lanceolate and glossy green in colour. They are covered in a waxy coating to reduce water loss. The long funnel-shaped some times fragrant yellow (less commonly apricot, sometimes white) flowers are in few-flowered terminal clusters. Inflorescence a terminal cyme. Its fruit is deep red-black in colour encasing a large seed that bears some resemblance to a "Chinese lucky nut".

#### Chemical Constituents:

Thevetin A and Thevetin B are the main Cardiac Glycosides found in yellow oleander seed. In addition to Thevetin A and B, Peruvidoside, Neriiflin, Thevetoxin, Peruvidosidic acid, Cerberin, Thevetoxin, Ruvidoside, Acetylneriifolin and Thevefolin are some of the other reported Glycosides, yellow oleander reported

to consist of 2 types of sugar- D- glucose and L-thevetose. Various Aglycones are also found in

*C. thevetia* are: Cannogenin, Cannogenol, Cannogenic acid, Digitoxigenin.

**Medicinal Value:**

*C. thevetia* is used as Biological pest control. The plant’s toxin have tested in experiments for uses in biological pest control. Its seed oil was used to make a “paint” with Antifungal, Antibacterial and Anti-termite properties.

- All parts of the *C. thevetia* plants are toxic to most vertebrates as they contain cardiac glycosides. The main toxin are the Cardenolides called Thevetin A and Thevetin B. Other include Peruvidoside, Nerifolin, Thevetoxin and Nuvidoside. These Cardenolides are not destroyed by drying or heating. They produce Gastric and Cardiotoxic effect.
- Antidotes for treatment include Atropine and Digoxin immune fabs (antibodies) and treatment may include oral administration of activated charcoal.
- Kaner is used in making medicines for leprosy, inflammation, Wound, Heart etc. Kaner whole plant is said to have Anticancer properties, help in leprosy, Malaria, Ringworm, Indigestion.

**Preparation of Extract in Cold Distilled Water Ezhilan. et.al, (1994):**

At first 250 gm of fresh leaves of each early described medicinal plants were separately taken and washed thoroughly with tap water and finally with distilled water to remove the outer unwanted material fixed on the upper parts of the leaves. Now they are dried in hot oven or incubator at 80°C for 2 days and the dried leaves of each medicinal plants were taken separately

in a mortar and were crushed with pestals. The crushed material now were taken separately in a mixer grinder to get fine powder and the powdered material were collected separately. Later on 2 grams of the powdered material of each plant were finally subjected to prepare the aqueous solution of 2 percent. These aqueous solution were taken separately in a bolted tubes and were kept in 5000 rpm centrifuge. Now they were rotated for 5 minutes and allowed the liquid material to settle down. The pure liquid settle at the bottom of the tube and erhanious matter come at the top of the liquid. They were removed and the pure extract was obtained. The pure extracts of all the five plants were allowed to settle completely for one day and after that a mixture of extract is prepared as such.

- Neem extract – 50 ml
- Thevetia extract – 5 ml
- Tulsi extract – 10 ml
- Calotropis extract – 7 ml
- Khus extract – 5 ml

These combination was processed in a separate conical flask of 250 ml and was shaken well. They were bolted tightly and was kept safely for antifungal bioassay.

**Experimental Finding:**

The experiment is carried out considering four pathogenic fungi which become prevalent on Makhana plant like *Alternaria* Spp., *Fusarium solani*, *Rhizoctonia fabi* and *Sclerotinia sclerotiorum*. All these four fungi were grown separately on seven days old culture of PDA media and were transferred to five different petri plates containing antifungal extracts. After seven days the result were recorded in diameter and sporulation were counted. All results were placed in table as such.

| S. No. | Concentration | Name of Pathogens      |             |                        |             |                         |             |                                 |             |
|--------|---------------|------------------------|-------------|------------------------|-------------|-------------------------|-------------|---------------------------------|-------------|
|        |               | <i>Alternaria Spp.</i> |             | <i>Fusarium solani</i> |             | <i>Rhizoctonia fabi</i> |             | <i>Sclerotinia sclerotiorum</i> |             |
|        |               | Mycelium diameter      | Sporulation | Mycelium diameter      | Sporulation | Mycelium diameter       | Sporulation | Mycelium diameter               | Sporulation |
| 1      | 5 ml.         | 5 mm                   | ++          | 15 mm                  | +++         | 8.1 mm                  | ++          | 10.6 mm                         | ++          |
| 2      | 10 ml.        | 2 mm                   | +           | 10.5 mm                | ++          | 6.3 mm                  | ++          | 8.4 mm                          | ++          |
| 3      | 15 ml.        | --                     | --          | 8.3 mm                 | +           | 4.1 mm                  | +           | 6.3 mm                          | +           |
| 4      | Control       | 40 mm                  | ++++        | 36.3 mm                | ++++        | 38.3 mm                 | ++++        | 46.1 mm                         | ++++        |

- + = Poor Sporulation
- ++ = Medium Sporulation
- ++++ = Excellent Sporulation
- = No growth and Sporulation

Cd - 5 % – 0.1238  
SE - 1 % – 0.367

**RESULT AND DISCUSSION:**

From the above table it is evident that almost all medicinal plants contained the antifungal ingredients. They are successfully controlling the growth and sporulation of pathogenic fungi. In this result, it has been observed that the extract shows potent antifungal activities in case of some pathogenic fungi and in case of other the effect is moderate. The *Alternaria* spp. Shows more inhibitions in comparison to *Fusarium* and *Sclerotinia*. However it has more powerful activities in case of *Rhizoctonia fabi*.

**CONCLUSION:**

After viewing the above result which is presented in table, it is concluded that, the extract prepared from the five medicinal plant could be able to control the spread of pathogenic fungi on Makhana plant and it will be proved the alternatives of the chemical fungicides formulated for the control of pathogenic fungi. The prophylactic spray of the extract on Makhana leaves will be helpful for control the *Alternaria* Leaf Blight and *Rhizoctonia* Stem decay. If it is possible, then why there should be the use of chemical fungicides which become harmful for

the health of human beings and further if the Makhana product may be found to be loaded with this harmful chemical gives bad impact on the foreign trade market. The antifungal constituent found inside the herbal source has no side effects and there greater mass becomes not harmful at any concentration. So it is suggested that the Government should promote the herbal industry and give priority to obtain the antifungal constituent from herbal source. There are two benefits from this are following:-

1. Bonafide health of human being.
2. Low cost of this chemical will help the poor farmer.

This finding is in accordance with the finding of Shrivastava and Lal (1997). They found the leaf extract of *Calotropis procera*, *Lantena camara*, *Ocimum basilicum* and *Azadirachta indica* stopped the mycelia growth of *Curvularia tuberculata* and *Alternaria alternata*. They also reported the maximum toxicity against spore germination. Further this result justifies the result of Bansal and Gupta (2000) who evaluated some plant extract against *Fusarium oxysporum* a wilt pathogen of Fenugreek. They have reported the inhibition of growth and sporulation by the extract of *Calotropis procera*, *Azadirachta indica*, *Atropa belladonna* and *Lantena camara*. This work is in accordance with the findings of Zewain et al, (2004) who studied the effect of fungicides and neem extract on the mycelia growth of *Sclerotinia sclerotiorum* and reported the mycelia growth only 2 mm in diameter. In this work the mycelia diameter of *Alternaria* has been recorded 2 mm at 5 ml dose.

From the present work, it can be concluded that the nature has provided the mankind a lot of facilities for their protection but there is need of proper search and investigation. Higher plants grow in nature and contain a lot of chemical constituents which can be applied to treat the infected plants. These chemicals work like commercial fungicides. As we know that these commercial chemicals by no means are useful for the health of mankind but skill we are applying these on our infected crops.

Finally we take these chemicals with the agricultural produce and get result in the form of different health disorders. If such type of research work is carried out and ecofriendly chemicals will be found out then it may be possible that our infected crops will be treated with the beneficial chemical which at any dose will be no harmful for our health. In this way we will be able in providing some beneficial aspects to the suffering society.

## REFERENCES

1. Bansal, R.K. and Gupta, R.K. (2000). Evaluation of plant extracts against *Fusarium oxysporum*, the wilt pathogen of Fenugreek, IPS, Vol.53(1):p.107-108.
2. Bansod, S. and Rai, M. (2008). Antifungal Activity of Essential Oils from Indian Medicinal Plants against Human Pathogenic *Aspergillus fumigates* and *A.niger*, World.j.Med.sc.,3(2):81-88
3. Bhatt, Meenakshi, Mishra, M., Singh, J.V. and Anita. Phytochemical and Antimicrobial studies of *Adhatoda zeylanica*, IJRAP,2010, 1(1),90-97.
4. Gerard Ezhilan, J. Chandrasekhar, V. and Kuruchev, v. 1994 effect of Six selected plant products and oil cakes on the sclerotial production and germination of *Rhizoctonia Solani* Ind. Phytopath

.47:183-85.

5. Jha, V. Barat, G.K. and Jha, U.N. (1991). Nutritional Evaluation of *Euryale ferox salisb.* (Makhana). Journal of Food science and Technology, 28(5):326-328.
6. Khan, J.A. and Tewari, S. (2011). A study on Antibacterial Properties of *Rosa indica* against various pathogens, Asian.j.plant sc. And Res.,1(1):22-30.
7. Mahadevan, A. (1970). Prohibitin and disease resistance. Phytopath.Z. Vol.68:p.73-80. Mannimegalai, V., Ambikapathy, V. and Paneer- Selvam, A. (2011).
8. Antifungal potentiality of some medicinal plant extracts against *Bipolaris Bipolaris Oryzae*. Asian Journal of plant Science and Research, 1(3):77-80.
9. Masood, N., Ahmed Chaudhry, A. and Perween, Tariq (2008). In vitro Antibacterial Activities of Kalonji, Cumin and Poppy seed, Pak.j. Bot.,40(1), 461-467.
10. Shrivastava, A.K. and Lal, B. (1997). Studies on biofungicidal properties of leaf extract of some plants. Ind. Phytopath.,50(3):408-411.
11. Zewain, Q. K., Bahadur, P., Sharma, P. (2004). Effect of fungicides and Neem extract on mycelia growth and mycelio genic germination on *Sclerotinia sclerotiorum*. Ind. Phytopath. 57(1):101-103.