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ORIGINAL RESEARCH ARTICLE

Evaluation of the Antifungal Activity of Nirgundi (*Vitex negundo*) against Early Blight of Tomato caused by *Alternaria solani* Fungi

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ABSTRACT

Introduction: Fungal pathogens are emerging as a major threat to agricultural productivity and food security worldwide. Although chemical fungicides are widely used to control plant diseases, their excessive application has resulted in environmental pollution, harmful effects on living organisms, and the development of pathogen resistance. In recent years, bio-pesticides have gained attention as a sustainable and eco-friendly alternative to conventional pesticides. Bio-pesticides are generally less hazardous, target-specific, biodegradable, and effective even in small quantities. Unlike broad-spectrum chemical pesticides, they minimize harm to beneficial organisms such as insects, birds, and mammals, while reducing environmental contamination. India's bio-pesticide industry has significant potential for growth due to increasing awareness about organic farming and the demand for safer agricultural practices.

Aim and objectives: this article discusses the importance of bio-pesticides made from drugs mentioned in classical Ayurveda texts and their visible effect as a bio-fungicide.

Material and Method: drug used here is Vitex Negundo (nirgundi) and its effects as a bio-fungicide is observed in in-vitro conditions against Alternaria Solani fungi causing early blight in tomatoes. Result: significant compared to the control group with antracol an chemical fungicide.

Discussion and Conclusion: • Among aqueous and hydro-alcoholic extracts, hydro-alcoholics shows more significant results for Alternaria solani whereas 5% and 10% aqueous extract shows zero percent efficacy and 15% aqueous extract showing only 5.50 percent efficacy against Alternaria solani. • It means that chemical pesticide used for treatment of Alternaria solani manifestation can be replaced with hydro-alcoholic extracts of Nirgundi (15%) as a bio-fungicide.

1. INTRODUCTION

Recently, it has been found that fungal pathogens are going to be one of the greatest threats to our calories crops, to control these pathogens fungicide is of great use, but increasing resistance and their further toxicity put us in an arms race against them. Pesticides known as fungicides either eradicate or stop the fungi's growth and their spores.[1]

Pesticides are a big umbrella under which fungicide is one. As these compounds are made with various chemical combinations they produce various ill effects to the living beings who are consuming it as well as to the environment.

India's bio-pesticide industry has a lot of room to grow. Dependence is a significant problem even though organic farming can feed an expanding population.

- Typically, bio-pesticides are less hazardous by nature than traditional pesticides
- Contrary to broad-spectrum, conventional pesticides, which may harm a variety of organisms, including birds, insects, and mammals, bio-pesticides often affect only the pest of interest and creatures that are closely related

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- Bio-pesticides frequently work in extremely small doses and frequently degrade quickly, reducing exposure levels and mainly avoiding the pollution issues brought on by conventional pesticides.[2]

Bio-pesticides are readily biodegradable and break down after use, posing little threat to people, food, or the environment. This avoids bioaccumulation and, thus, issues with environmental pollution brought on by synthetic pesticides.

India is the fourth largest producer of tomatoes worldwide with a production of 18.7 million tones, with an annual growth rate of 30% in 3 years.[3]

2. MATERIALS AND METHODS

2.1. Aim

The aim of the study was to study the antifungal effect of *Nirgundi* aqueous and hydro-alcoholic extract (*Vitex negundo*) on *Alternaria solani* fungi in laboratory as *in vitro* study.

2.2. Preparation of Extract of Nirgundi

2.2.1. Aqueous extract

- Approximately 100 g of aqueous extract was prepared, and for that, 1 kg of *Nirgundi patra churna* was taken, which was mixed with 20 L of filtered water
- The solution was then put on a rotary shaker for 18 h and later for 6 h on standby Filtration of the extract through Whatman No. 1 filter paper.
- The filtered extract than transferred china dish for water bath evaporation technique. Extract was collected after being dried.

2.2.2. Hydro-alcoholic

- To prepare 100 g of hydro-alcoholic extract 1 kg fine *churna* of *Nirgundi* was required, which was mixed with equal amount of filter water and alcohol that is 10 L each
- Solution was then put on rotary shaker for 18 h and later for 6 h on standby
- Filtration of the extract through Whatman No. 1 filter paper
- The filtered extract than transferred china dish for water bath evaporation technique
- Collection of the extract was done after being dried.

2.3. Plan of Study

Alternaria solani culture is procured and isolated from infected tomatoes. Procured samples were multiplied in laboratory.

Nirgundi bought to the laboratory for standardization.Hydro-alcoholic and aqueous extract of nirgundi was prepared in concentration of 5, 10 and 15 percent for *in-vitro* study.

Plan of study as a title of Flow chart 1 mentioned below.

2.4. Materials

- *A. solani* culture
- *Nirgundi* extract:
  - Water based (5, 10, 15% concentration)
  - Hydro-alcoholic (5, 10, 15% concentrations)

Petri dish with potato dextrose medium

2.5. Methods (*In Vitro*)

2.5.1. Type of study

- Experiment design: Controlled randomized blocked design
- No of treatment: 8
- No. of replication: 3.
- Place of study: Experiment was conducted at Department of Pathology, Durgapura, Rajasthan Agricultural Research Institute Jaipur

The sample was divided into two parallel groups

- Group 1: Was divided into 3 sub-groups and were sprayed with *Nirgundi* aqueous-based extract (5, 10 and 15% concentration) tested for bio-fungicide
- Group 2: Was divided into 3 sub-groups and were sprayed with *Nirgundi* hydro-alcohol based extract (5, 10 and 15% concentration), tested for bio-fungicide
- Positive control group: Was tested with antracol fungicide
- Negative control: Inoculated with *A. solani* fungi.

The whole study was carried out mainly in 3 steps:

- Extract preparation of nirgundi
- Inoculation of extract into samples
- Result analysis.

2.6. Extract Preparation of Nirgundi

Extract needed to be prepared was aqueous and hydro-alcoholic in concentration of 5–10 and 15%.

2.6.1. Aqueous extract preparation

- For 5% extract, 5 g of dry aqueous extract was mixed with 95 mL of water
- For 10% extract, 10 g of dry aqueous extract was mixed with 95 mL of water
- For 15% extract, 15 g of dry aqueous extract was mixed with 95 mL of water.

2.6.2. Hydro-alcoholic extracts preparation

- For 5% extract, 5 g of hydro-alcoholic extract was mixed with 95 mL of an equal amount of water and alcohol
- For 10% extract, 10 g of hydro-alcoholic extract was mixed with 95 mL of an equal amount of water and alcohol
- For 15% extract, 15 g of hydro-alcoholic extract was mixed with 95 mL of an equal amount of water and alcohol

2.6.3. Inoculation of extract into samples

- Petri-dishes were prepared for the experiment using potato-dextrose medium in a sterilised environment.
- Each sample had three replicas, and an average value had been calculated.
- These dishes were inoculated with samples of *Nirgundi* aqueous extract (5, 10, 15%) and *Nirgundi* hydro-alcoholic extracts (5, 10, 15%), along with a control group (propineb 70%) and a negative control (no treatment given) group
- Inoculation of dishes with *A. solani* culture
- Dishes were covered to prevent external infection and kept in dry and sterilized environment
- Time taken to cover full plate in the negative control is 14 days, which was the time of observation. Dishes were inoculated with samples and *A. solani* and observed after 15 days
- Percentage inhibition was calculated using Vincent formula.

$$\text{Vincent formula} = \frac{C - T}{C} \times 100$$

C = Total diameter of the Petri dish (9 cm)
T = Diameter covered by *A. solani* infection.

3. RESULTS

In this study, it was observed that the aqueous and hydro-alcoholic extract preparations of *Nirgundi* are showing significant bio-fungicidal activity against *A. solani* fungi. Out of all the samples, hydro-alcoholic extract preparation is most significant, showing results similar to the chemical fungicide propineb 70% (Table 1).

4. DISCUSSION

Chemical pesticides have a range of outcomes. 200 deaths are thought to be caused each year in the USA by insecticide poisoning. According to the World Health Organization, there are 25 million incidents of pesticide poisoning each year, and 20,000 people die as a result in underdeveloped nations.[4]

The first commercially available bio-insecticide was produced in year 1930, and the majority of which are entomopathogenic hypomycete fungi. Consequently, bio-insecticides have been most successful in warm, humid conditions.[5] Bio-pesticides are made from insects, microbes, and plants. Plant and animal-based substances have been utilized to treat ailments in humans, animals, and crops. Many people all across the world practice depending on nature to heal it.[6]

India's bio-pesticide industry has a lot of room to grow. Dependence is a significant problem, even though organic farming can feed an expanding population typically, bio-pesticides are less hazardous by nature than traditional pesticides.

Contrary to broad-spectrum, conventional pesticides, which may harm a variety of organisms, including birds, insects, and mammals, bio-pesticides often affect only the pest of interest and creatures that are closely related.

Bio-pesticides frequently work in extremely small doses and frequently degrade quickly, reducing exposure levels and mainly avoiding the pollution issues brought on by conventional pesticides.[2]

The Asian market offers a huge opportunity for bio-pesticides as the mega-economies of China and India boost their use of bio-pesticides.[7] *A. solani* is a fungal pathogen that produces a disease in tomato and potato plants called early blight. In addition to producing recognizable "bulls eye" – patterned leaf spots, the pathogen can also cause tuber blight on potatoes, fruit rot on tomatoes, and stem lesions. Even though they are called "early," foliar ailments typically affect older leaves.[8]

A. solani is a deuteromycete with a polycyclic life cycle. *A. solani* uses conidia for asexual reproduction. *A. solani* is typically regarded as a necrotrophic pathogen, meaning that it consumes the dead plant cell material after killing the host tissue using toxins and enzymes that break down cell walls.[9]

Antracol is a fungicide used for early blight and late blight in tomatoes. This product may cause respiratory sensitization. This product may cause sensitization from prolonged skin contact. This product is presumed to cause target organ damage from repeated oral exposure at high doses. It is also toxic to aquatic organisms can cause biomagnification in aquatic animals, which eventually reaches to humans.[10]

4.1. Nirgundi

It has been found that this drug was initially used as medicine from *Samhita Kala*. In *Charaka Samhita*, *Nirgundi* has been mentioned under the topic of *Vishaghna* and *Krimighna Mahakashaya*. It was used in *Kshira Vaivarnya*, *Yonivyapad Chikitsa*, *Krimi* and *Visha Chikitsa* etc. In *Sushruta Samhita*, two varieties of *Nirgundi* have mentioned, that is, (a) *Nirgundi* and (b) *Sita Sindhuvar*, where commentator of *Sushruta,* that is, *Dalhan,* says that *Nirgundi* means *Nilapushpa* and *Sita Sindhuvar* means *Shweta* Variety. Here, the formal drugs are listed in *Surasadi Gana* and later in *Shwasaghna*, *Rakshoghna* and *Vrana Shodhana*.

The plant is bitter, acrid, thermogenic, antihelminthic, expectorant, carminative, digestive, stomachic, anodyne, anti-inflammatory, antiseptic, cephalic, alterant, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary and tonic. The roots are considered tonic, febrifuge, expectorant and useful in cephalalgias, otalgia, arthritis, inflammations, dyspepsia, colic, rheumatism, leprosy, verminosis, flatulence, dysentery, urinary disorders, wounds, ulcers, bronchitis, cough, malarial fever, hemorrhoids, dysmenorrhea, leprosy, skin diseases and general debility. The leaves are aromatic, tonic, vermifuge and useful in rheumatism, arthritis, catarrhal fever, cephalagia, sprains, orchitis, syphilis, inflammations and ulcers. An oil is prepared with the juice of leaves is applied to sinuses, scrofulous sores, wounds, ulcers and gangrenous wounds. The bark is useful in odontalgia, verminosis and ophthalmic disorders. The flowers are useful in diarrhea, cholera, fever, hemorrhage, hepatopathy and cardiac disorders. Leaves and bark are useful in scorpion stings.[11]

Leaves and roots constitute the drug. The leaves are applied to rheumatic swelling of joints, in sprains etc. The leaves are considered tonic and vermifuge. The leaves are smoked for the relief of headache and catarrh. The decoction of leaves and the vapors are employed in bath for the treatment of febrile, catarrhal, and rheumatic affections. The root possesses tonic, febrifugal, expectorant, and diuretic properties. They are used in dyspepsia and rheumatism.[12]

5. CONCLUSION

From the current work, the following conclusions may be made:

- The usage of pesticides, such as propineb, has expanded daily in tandem with the green revolution
- Chemical pesticides are found in all edible things, including tomatoes. To prevent them from various microorganisms infestations like *A. solani,* causing early blight in tomatoes. However, these pesticides have hazardous effects on human health
- Bio-pesticides are the best approach against these hazardous chemical pesticides, protecting the plants from microbial infestations effectively, and meanwhile non-hazardous for human and animal life.

5.1. *In Vitro* Study

- The present *in vitro* study shows that 15% hydro-alcoholic extract shows maximum efficacy against *A. solani* (48.00%)
- Propineb 70% used as a positive control group shows 43.78% efficacy, followed by 10% hydro-alcoholic extract of *Nirgundi*

with 22.20% efficacy

- Among aqueous and hydro-alcoholic extracts, hydro-alcoholics show more significant results for *A. solani* whereas 5% and 10% aqueous extract shows 0% efficacy, and 15% aqueous extract showing only 5.50% efficacy against *A. solani*
- It means that chemical pesticides used for the treatment of *A. solani* manifestation can be replaced with hydro-alcoholic extracts of *Nirgundi* (15%) as a bio-fungicide

In the absence of 15% hydro-alcoholic extract of *Nirgundi,* 10% of hydro-alcoholic extract can be used.

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7. AUTHORS' CONTRIBUTIONS

All authors give equal contribution in making of this manuscript.

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Nil.

9. ETHICAL STATEMENT

Ethical approval was not required for this study as it is a experimental study.

10. CONFLICTS OF INTERESTS

The authors declare no conflicts of interest regarding the publication of this paper.

11. DATA AVAILABILITY STATEMENT

The data analyzed in this review were obtained from publicly available sources, including peer-reviewed articles, observational studies, and surveys accessible through databases.

12. DISCLAIMER/VIEW AND OPINIONS

The opinions expressed in this article are solely those of the authors and do not reflect the views of the International Research Journal of Ayurveda and Yoga or its editorial board.

13. AI-USE DECLARATION

The authors declare that no generative AI tools were used to create scientific content, interpret data, or draft any sections of this manuscript. AI-based tools were used solely for minor language and grammar refinements to improve clarity and readability. All scientific content, analysis, and conclusions remain the sole responsibility of the author.

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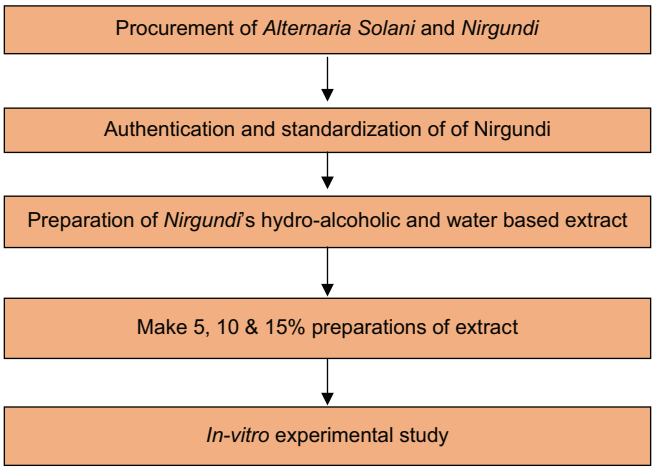
Table 1: Comparing the mean fungicidal activity of aqueous and hydro-alcoholic extract of *Nirgundi* along with propineb 70%

S. No.	Group	Sub-groups	Average mycelial growth inhibition %
1.	Aqueous	5% preparation	0.00 (0.00*)
		10% preparation	0.00 (0.00)
		15% preparation	5.50 (13.56)
2.	Hydro-alcoholic	5% preparation	5.90 (14.04)
		10% preparation	22.20 (28.10)
		15% preparation	48.00 (43.83)
3.	Propineb 70% WP (positive control)	-	43.78 (41.41)
4.	Uninoculated (negative control)	-	0.00 (0.00)
CD			1.41
SE (m)			0.47
CV			4.57

"*" stands for arcsine value, which is used when the difference between various observed value is larger. Since the average mycilinated values vary from zero to 48.63 arcsine value is used. The formula used is Vincent formula



Flow chart 1: Plan of study