Advances in Bioresearch
Adv. Biores., Vol 16 (5) September 2025: 209-215

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.5.209215

Advances in Bioresearch

ORIGINAL ARTICLE

A Study on Endophytic Fungi in Peanut Seeds of Telangana State and their Biocontrol by using Extracts of *Tamarindus indica* and *Adathoda vasica*

R. Venkat Ramchandar
Department of Microbiology, Tara Government College (Autonomous), Sangareddy, Telangana, India-502001
***Corresponding Author:** Email: 23venkat73@gmail.com
ORCID: 0009-0007-4130-7219

ABSTRACT
Peanut crop cultivation in Telangana state increased to 3,88,745 acres in 2021-22, with vulnerability to various pathogenic fungi that can impact plant health and yield. Aspergillus flavus, a common mold in tropical regions, produces aflatoxins, posing a significant risk to peanut crops, especially during poor post-harvest storage conditions. Aflatoxins are dangerous to both humans and animals, linked to cancer and death when ingested in large amounts, and are found not only in peanuts but also in various other foods. Aflatoxins are the most potent mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus, with 60% of Aspergillus flavus strains producing aflatoxins. Fumonisins, another type of fungal toxin, are produced by several species of the genus Fusarium, in addition to Alternaria alternate f.sp. lycopersici. In this study we isolated and identified endophytic fungi from peanut seeds collected from various regions of Telangana state by colony morphology and microscopy. We also studied antifungal effect of ethanol extracts of leaves of Tamarindus indica and Adathoda vasica for biocontrol of endophytic fungi by poison food method. In vitro assay by poison food method showed that ethanol extract of Adathoda vasica (AVE) exhibited percentage inhibition ranging from 75% to 89%, whereas the ethanol extract of Tamarindus indica (TIE) showed inhibition ranging from 75% to 90.5% against different endophytic fungi. Our findings suggest that both ethanol extracts of leaves of Tamarindus indica and Adathoda vasica could be potential sources of natural antifungal agents. These results indicate a promising avenue for biocontrol strategies using phytoextracts to control fungal contaminants and aflatoxins in peanuts.
Keywords: *Endophytic fungi, pea nut seeds, poison food method, Tamarindus indica, Adathoda vasica*

Received 20.04.2025 Revised 09.07.2025 Accepted 24.09.2025

How to cite this article:
R. Venkat Ramchandar. A Study on Endophytic Fungi in Peanut Seeds of Telangana State and their Biocontrol by using Extracts of *Tamarindus indica* and *Adathoda vasica.* Adv. Biores., Vol 16 (5) September 2025: 209-215.

INTRODUCTION

In Telangana state, Pea nut crop is grown to an extent of 0.21 M ha with a production of 0.355 Mt and productivity of 1320 kg ha^{-1}. Cultivation of Peanut crop in 2021-22 increased to 3,88,745 acres [1]. The peanut (*Arachis hypogaea*) crop is vulnerable to a wide range of pathogenic fungi that can severely impact plant health and yield. These fungal pathogens are capable of invading all parts of the plant—including the roots, stems, leaves, and pods—either directly or through wounds caused by mechanical damage, pests, or environmental stress. Such infections can lead to various plant diseases and contribute to post-harvest contamination, particularly with mycotoxin-producing fungi like *Aspergillus* spp. Several researchers isolated many fungi such as *Aspergillus niger, Aspergillus flavus, Alternaria dianthicola, Curvularia lunata, Curvularia pellescens, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseolina, Rhizopus stolonifer, Penicillium digitatum* and *Penicillium chrysogenum* etc. [2,3]. *Aspergillus flavus* is a common mold prevalent in tropical and subtropical regions, and it produces aflatoxins as secondary metabolites, particularly under conditions of poor post-harvest storage. Peanuts are highly susceptible to aflatoxin contamination, especially when stored improperly, creating ideal conditions for fungal growth and toxin production [4]. Peanuts are one of main crops that are vulnerable to mycotoxins,

particularly aflatoxins, these natural contaminants are dangerous to both humans and animals and linked to cancer and death when ingested in large amounts. Aflatoxins are found not only in peanuts, but also in many other foods, including corn, milk, eggs, meat, nuts, almonds, figs and spices, corn, cotton seed etc. Aflatoxins are also sometimes detected in milk, cheese, eggs and meat when animals ingest contaminated feed.

Aflatoxins are most potent mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* and other fungi *A. niger, A. oryzae, A. ochraceus, Penicillium citrinum* etc. It has been found that 60% strains of *Aspergillus flavus* produce aflatoxins. *Aspergillus flavus* and *Aspergillus parasiticus* produce Aflatoxin B1 and B2, G1and G2 [5,7]. Fumonisins are fungal toxins produced by several species of the genus *Fusarium*, especially *Fusarium verticillioides*, *Fusarium proliferatum*, and *Fusarium nygamai*, in addition to *Alternaria alternate* f.sp. *lycopersici* [6,7]. In this study we isolated and identified endophytic fungi from peanut seeds collected from various regions of Telangana state by colony morphology and microscopy. We also studied antifungal effect of ethanol extracts of leaves of *Tamarindus indica* and *Adathoda vasica* for biocontrol of endophytic fungi by poison food method.

MATERIAL AND METHODS

Collection of fresh Pea nut Seeds

About 250 grams/ 500 grams of Peanut seeds are collected from farmers of various districts of Telangana state through students of GDC Kukatpally in clean polythene covers. Groundnut seeds were collected from farmers of selected districts (Sangareddy, Zaheerabad, Siddipet, Medchal, Mahabubnagar, Nagarkurnool) of Telangana state and the fungal flora associated with peanuts were isolated and identified by inoculating seeds in potato dextrose agar medium in petri dishes. Three samples were collected from each farmer. Approximately one kg of pods of peanuts were collected from each farmer, brought to the laboratory in polythene bags. Pods were then hand shelled and divided into sub samples. These samples were used for examination of fungal flora associated with peanuts.

Surface Sterilization of peanut seeds and inoculation on PDA medium

Pea nut seeds are dipped in 70% ethanol and placed for five minutes for surface sterilization in petri plates. Then cleaned in sterilized distilled water and placed on potato dextrose agar (PDA) medium in petri plates. In each Petri plate, 6 seeds are placed. Plates are incubated at 28^{o}C in incubator for 5 days and observed every day for the growth of fungi. Colonies formed around each seed are studied for colony morphology and microscopy for identification of fungal genera. The percent incidence of each fungal species in peanut seeds was calculated as follows [8,9].

$$Fungal\ speceis\ (\%) = \frac{No\ of\ seeds\ colonized}{Total\ no\ of\ seeds\ placed} X\ 100.$$

Microscopic Examination of fungal isolates

On clean grease free slides, a drop of Lactophenol cotton blue is placed. Fungal isolate from PDA medium is taken with sterile forcepsand placed in Lactophenol blue and gently spread with needles and covered with cover slip and sealed. Slide is examined under Olympus binocular research microscope in 100x and 400X magnification for identification of fungi [10].

Extraction of leaf Extracts from *Adathoda vasica* and *Tamarindus indica*

Leaves of *Adathoda vasica* and *Tamarindus indica* are collected from garden of GDC, Kukatpally. Leaves are washed twice and air dried for one week.Dried leaves are powdered using a mixigrinder,30grams of powder is placed in 300 ml water and another 30 grams in 70% ethanol and placed on shaker for 3days. Suspensions are filtered through Whatman No.1 filter paper and stored at 4^{o}C for further use. Extracts also dried by placing in hot air oven in small containers at 45^{o}C for 48 hours. Dried powder is used in antifungal activity tests (AFT) [11,12,13].

Anti- fungal activity assay of phytoextracts by poison food method

Potato dextrose agar medium [150ml) is prepared, sterilized and mixed with 150mg ofextracts (1mg/ml) and poured into Petri plates. Potato dextrose agar medium without phytoextracts also kept as negative control. After solidification and one day incubation of plates for sterility test, each plate in center is inoculated with 5mm fungal mycelial mat culture disc using a sterilized cork borer. Control plates also inoculated with 5mm disc of fungal mat culture. All plates are incubated in incubator at 28^{0}C for 5 days. After incubation colony diameter of fungi is measured using a scale both in extract plates and negative control plates [14]. Percent inhibition was calculated by following formula.

$$PI = \frac{C - T}{C} X\ 100$$

C= Diameter of fungal colony in control plate

T= Diameter of fungal colony in extract plate.

Phytochemical Analysis:
Aqueous and ethanol extracts of *Adathoda vasica* and *Tamarindus indica* are screened for various plants secondary metabolites such as alkaloids, resins, tannins and phenolic compounds, saponins etc [15,16].

Analysis of Results
All experiments were done three times in triplicates and mean values are taken for analysis.

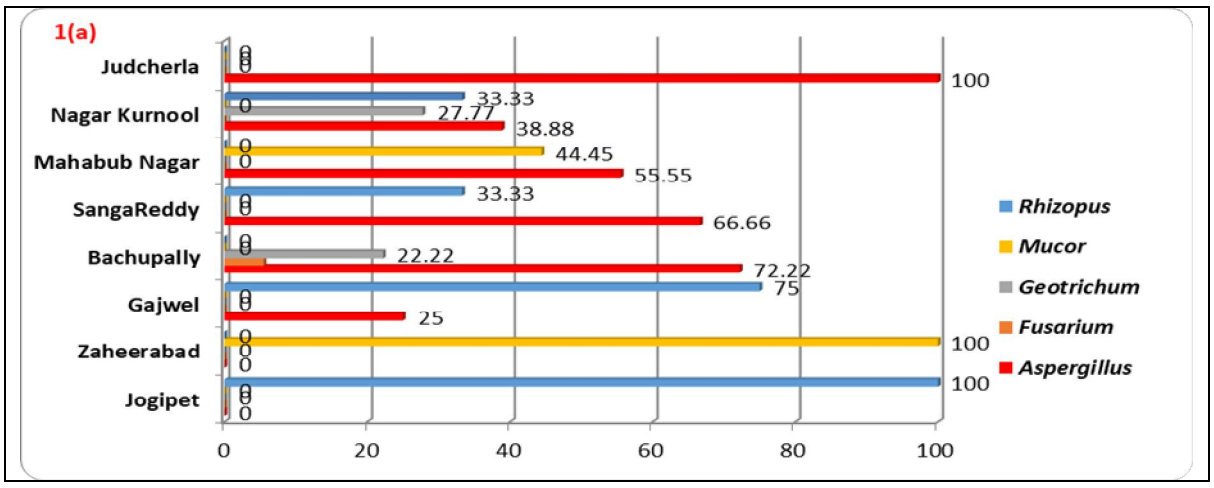
RESULT AND DISCUSSION

This study found that peanuts collected from various regions of Telangana contains *Aspergillus, Rhizopus, Mucor, Geotrichum and Fusarium* species. The different fungal genera and their distribution in peanuts collected from various districts were shown in Table 1 and Figure.1(a) & 1(b). This study indicated that peanuts collected from various regions of Telangana have *Aspergillus, Rhizopus, Mucor, Geotrichum and Fusarium* species. Pea nut Seeds from Jogipet has *Rhizopus* (100%) only, Peanuts from Zaheerabad has *Mucor* (100%). Peanuts from Gajwel has 75% *Rhizopus* spp. and 25% *Aspergillus* spp. Peanut seeds from Bachupally has *Aspergillus* (72.22%), *Rhizopus* (22.22%) and *Fusarium* (5.5%). Peanut seeds from Sangareddy have *Rhizopus* (33.33%), *Aspergillus* (66.66%). Peanut seeds from Mahabubnagar have *Aspergillus* (55.55%) and *Mucor* (45.45%). Peanut seeds from Nagarkurnool have *Geotrichum* (27.77%), Aspergillus (38.88%), Rhizopus (33.33%). Peanut seeds from Jadcherla have only Aspergillus (100%) species.

Table 1. Endophytic fungal isolates identified from peanut seeds.

S. No	Location /Place	District	No. of peanut seeds placed on PDA medium	Fungi	Distribution (%) of Fungi in peanuts of different districts of Telangana
1	Jogipet	Sangareddy	18	*Rhizopus*(18seeds)	*Rhizopus* (100%)
2	Zaheerabad	Zaheerabad	18	*Mucor* (18 seeds)	*Mucor* (100%)
3	Gajwel	Siddipet	18	*Rhizopus*(14seeds) *Aspergillus*(4Seeds)	*Rhizopus* (75%) *Aspergillus (*25%)
4	Bachupally	Medchal	18	*Aspergillus* (13 Seeds), *Rhizopus* (4 seeds) *Fusarium* (One seed)	*Aspergillus* (72.22%) *Rhizopus* (22.22%) *Fusarium* (5.5%)
5	Sangareddy	Sangareddy	18	*Rhizopus* (6 seeds) *Aspergillus* (12 seeds)	*Rhizopus* (33.33%) *Aspergillus* (66.66%)
6	Mahabubnagar	Mahabubnagar	18	*Mucor* (8 seeds), Aspergillus (10 seeds)	*Mucor (*44.45%) *Aspergillus* (55.55%)
7	Nagar Kurnool	Nagarkurnool	18	*Aspergillus* (7seeds), *Rhizopus* (6 seeds) *Geotrichum (*5seeds)	*Geotrichum* (27.77%) *Aspergillus* (38.88%) *Rhizopus* (33.33%)
8	Jadcherla	Mahabubnagar	18	*Aspergillus* (18 seeds)	*Aspergillus* (100%)

***The number and percentage distribution are mean value of three replicates.**



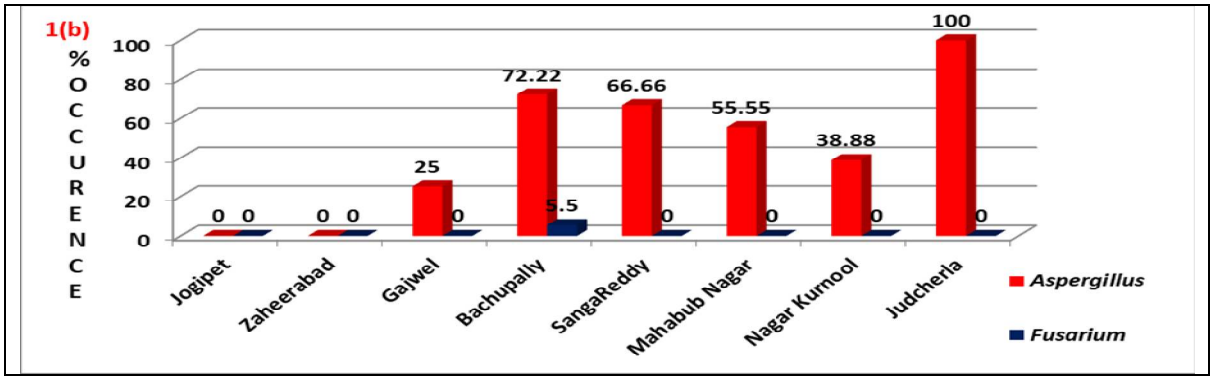


Figure.1(a): Percentage (%) occurrence of different endophytic fungi in peanuts of different locations of Telangana State. Figure 1(b). Percentage occurrence of mycotoxigenic fungi (*Aspergillus and Fusarium* spp) in peanuts of different locations in Telangana state.

In vitro poison food test proved that ethanol extracts of *Adathoda vasica* and /or *Tamarindusindica (*TI) has proved as potential antifungal compounds. *In vitro* poison food method revealed that ethanol extract of *Adathoda vasica* (AVE) showed percentage inhibition of 75% to 89%for different endophytic fungi. Ethanol extract of *Tamarindus indica* (TIE) showed percentage inhibition of 75% to 90.5% for different endophytic fungi (Table 2 and Figure 2, 3 and 4).

Table 2. Antifungal activity test (AFT) of phyto extracts by poison food method

S. No	Fungi	Diameter of Fungal colony in mm in Control PDA Plates(C)	Diameter of Fungal colony in mm in PDA medium containing ethanol extract of leaves of *Adathoda vasica*(T)	Percentage inhibition (PI)	Diameter of fungal colony in mm in PDA medium containing ethanol extract of leaves of *Tamarindus indica*(T)	Percentage inhibition (PI)
1	*Aspergillus*	36	9	75%	8	77.77%
2	*Rhizopus*	85	9	89%	8	90.58%
3	*Mucor*	80	10	87.5%	11	86.25%
4	*Fusarium*	40	8	80%	7	82.5%

***The diameter of fungal colonies is mean value of three replicates.**

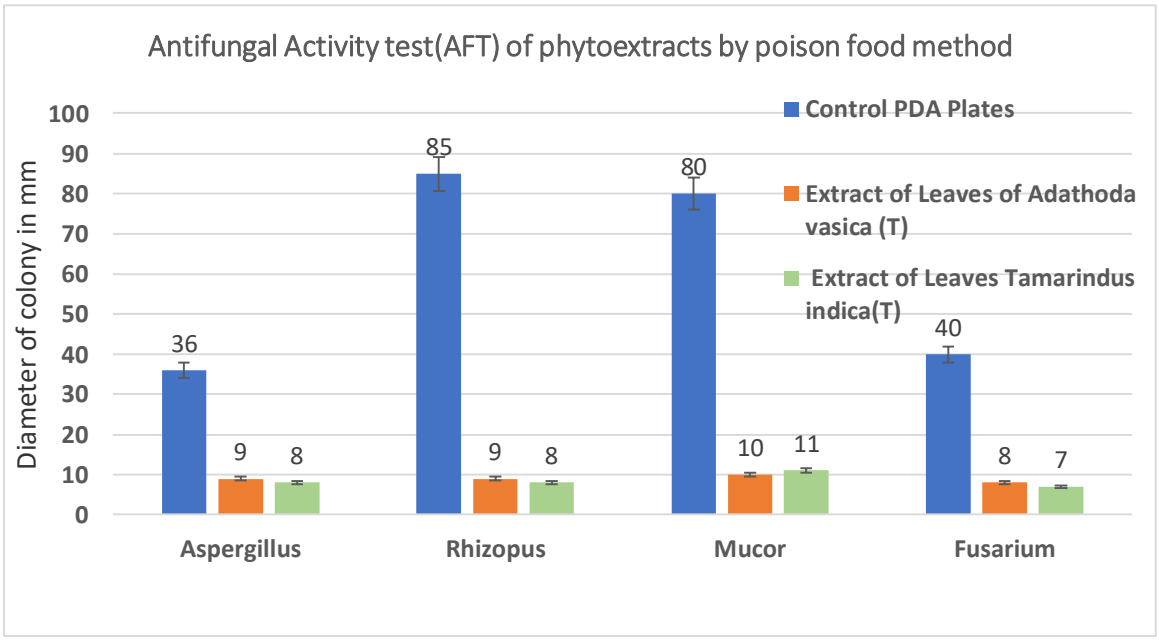


Figure 2. Antifungal activity test of ethanol extracts of leaves of *Adathoda vasica* and *Tamarindus indica*

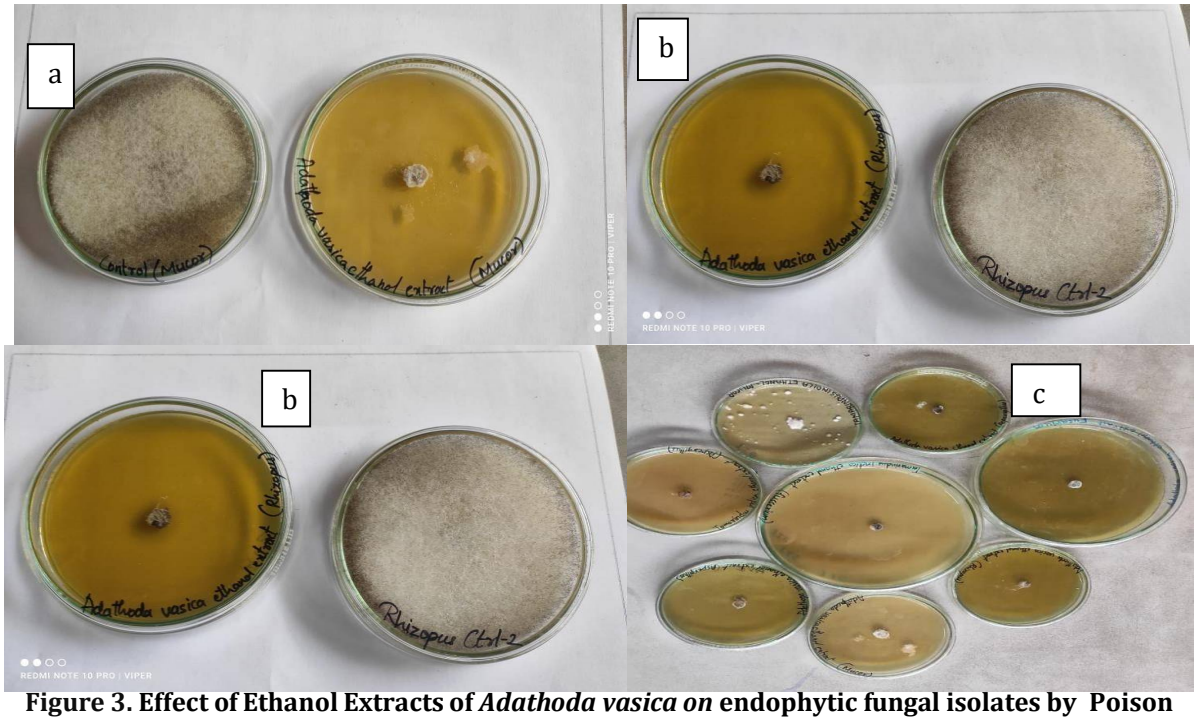


Figure 3. Effect of Ethanol Extracts of *Adathoda vasica on* endophytic fungal isolates by Poison Food Method (a*) Mucor* (b) *Rhizopus* (c) *Aspergillus, Rhizopus, Mucor, Fusarium*

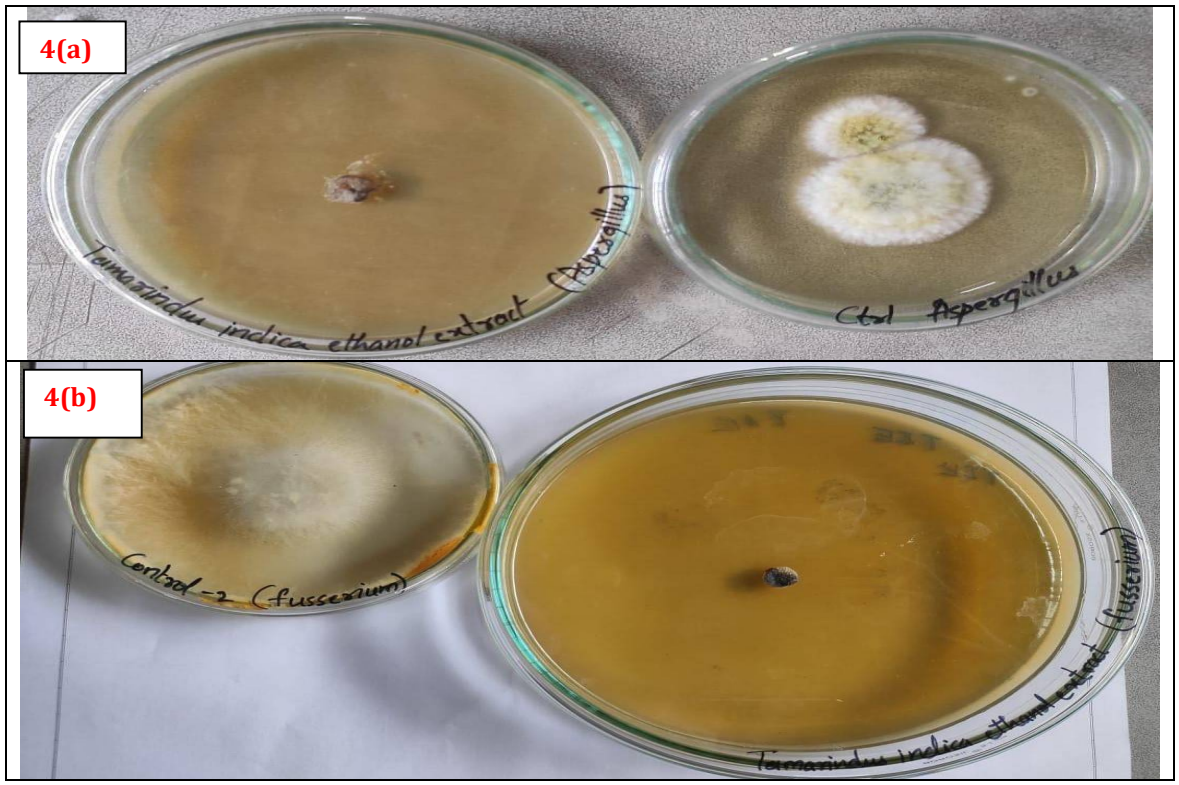


Figure. 4(a). Effect of ethanol extract of leaves of *Tamarindus indica* on *Aspergillus* and 3.4(b). Effect of ethanol extracts of leaves of *Tamarindus indica* on Fusarium species by poison food method.

Preliminary phytochemical analysis of ethanol and aqueous extracts of leaves of *Tamarindus indica* and *Adathoda vasica* revealed presence of various secondary metabolites such as alkaloids, tannins, phenolic compounds, saponins, terpenoids, coumarins, resins etc. The results were shown below in Table 3.

Table 3. Phytochemical Analysis of Ethanol and aqueous extracts of *Tamarindus indica* and *Adathoda vasica*

S. No	Phytochemical	Ethanol Extract Of *Tamarindus indica* (TIE)	Aqueous extract of *Tamarindus indica* (TIA)	Ethanol extract of *Adathoda vasica* (AVE)	Aqueous extract of *Adathoda vasica* (AVA)
1	Alkaloids	Positive (+)	Positive (+)	Positive (+)	positive (+)
2	Indole Alkaloid	Positive (+)	Negative (-)	Positive (+)	Positive (+)
3	Tannins, Phenolics	Positive (+)	Positive (+)	Positive (+)	Positive (+)
4	Saponins	Positive (+)	Positive (+)	Positive (+)	Positive (+)
5	Terpenoids	Positive (+)	Positive (+)	Positive (+)	Positive (+)
6	Coumarins	Positive (+)	Positive (+)	Positive (+)	Positive (+)
7	Resins	Positive (+)	Positive (+)	Positive (+)	Positive (+)
8	Quinones	Positive (+)	Positive (+)	Negative (-)	Negative (-)
9	Flavonoids	Negative (+)	Negative (-)	Positive (+)	Negative (+)

This study indicated that peanuts collected from various regions of Telangana have five endophytic fungi. These are *Aspergillus, Rhizopus, Mucor, Geotrichum and Fusarium.* Peanut seeds from Jogipet and Zaheerabad are absolutely free from *Aspergillus* and *Fusarium.* Peanuts collected from Gajwel region have 75% *Rhizopus spp.* and 25% *Aspergillus species.* Peanut seeds from Jadcherla have *Aspergillus* (100%). Keeping in view of occurrence of Aspergillus species in peanuts, this study revealed that Jogipet and Zaheerabad regions are more safe regions. Gajwel (Siddipet) and Nagarkurnool is very less risk regions. Mahabubnagar and Bachupally (Medchal) and Sangareddy regions are high risk regions. Whereas Jadcherla seems to be very high-risk region amongst all regions.

Ethanol extracts of *Adathoda vasica* and /or *Tamarindus indica (*TI) has proved as potential antifungal compounds. *Adathoda vasica* (AV) extracts or *Tamarindus indica* extracts can be used as amendment to soil to prevent growth of fungi such as *Aspergillus* spp and to reduce aflatoxin content in peanut seeds. Aflatoxins, produced by *Aspergillus* species are responsible for origin of digestive system cancers such as liver cancer, buccal cancer, oesophageal cancer and stomach cancer etc [17-19]. In our study aqueous extracts of AV and TI also showed antifungal activity. This indicate that addition of leaf powder of AV and TI may also control pathogenic fungi in peanuts and reduce fungal contamination of peanuts and reduce aflatoxin content in peanuts. Further Research such as soil amendment study requires time and is in progress.

CONCLUSION

Extracts of *Adathoda vasica* (AV) and *Tamarindus indica* (TI) possess significant antifungal activity to control phytopathogenic fungi. They can be used as natural botanical antifungal agents to control phytopathogenic fungi instead of copper based chemical pesticides in agriculture.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to Dr. N. Alivelu Mangamma, Principal, Government Degree College, Kukatpally, Hyderabad for providing Laboratory support and facilities during this research work and Dr. M. Praveena, Principal, Tara Government College (Autonomous) for support and encouragement.

REFERENCES

1. Telangana At a Glance, (2022). Directorate of Economics and Statistics, Government of Telangana. https://ecostat.telangana.gov.in/publication_archives.jsp
2. Al-Askar AA, Rashad EM, Moussa Z, Ghoneem KM, Mostafa AA, Al-Otibi FO, Arishi AA, Saber WI. A novel endophytic *Trichoderma longibrachiatum* WKA55 with biologically active metabolites for promoting germination and reducing Mycotoxinogenic fungi of peanut. Frontiers in Microbiology. 2022 Mar 10;13: 772417. DOI: 10.3389/fmicb.2022.772417
3. El-Zefzaf HM. (2020). Fungi associated with peanut seeds and their extracellular enzyme activities under the influence of physical factors. Asian J. Appl. Sci. 13:144-51. DOI:10.3923/ajaps.2020.144.151
4. Janardhan A, Subramanyam D, Praveen A, Reddi M, Narasimha G. (2011). Aflatoxin impacts on germinating seeds. Ann. Biol. Res. 25;2(2):180-8
5. https://www.researchgate.net/publication/216034385_Aflatoxin_Impacts_on_Germinating_Seeds
6. Ditta YA, Mahad S, Bacha U. (2019). Aflatoxins: Their Toxic Effect on Poultry and Recent. Mycotoxins: Impact and Management Strategies. 28:12. http://dx.doi.org/10.5772/intechopen.80363
7. Samal S, Rai S, Upadhaya RS. (2023). Endophytic Fusarium and their association with plant growth. In Microbial

endophytes and plant growth.(pp. 259-268). Academic Press. https://doi.org/10.1016/B978-0-323-90620-3.00002-7
8. Mycotoxins, Toxins found in food infected by certain Molds or fungi, US Food and Drug Administration, https://www.fda.gov/food/natural-toxins-food/mycotoxins
9. Adithya G, Rajeshwari B, Keshavulu K, Sudini H, Swathi Y. (2017). Mycoflora associated with groundnut seeds collected from selected groundnut growing districts of Telangana State, India. International Journal of Current Microbiology and Applied Sciences. 2017 Jul 10;6(7):4335-42. DOI: https://doi.org/10.20546/ijcmas.2017.607.451
10. Tobin-West MD, Dimkpa SO, Osakwe JA. (2018). Isolation and identification of fungi associated with raw groundnut seeds sold at four major markets in Port Harcourt Metropolis, Rivers State. Journal of Biology, Agriculture and Healthcare. 8(6):29-35. https://core.ac.uk/reader/234662549
11. Bhargava B, Tandon N. (2015). Standard operating procedures for fungal identification and detection of antifungal resistance. Indian Council of Medical Research (ICMR): Karnataka, India.;7(9):27-44. https://www.icmr.gov.in /icmrobject/custom_data/pdf/resource-guidelines/Mycology_SOP_2nd_Ed_2019.pdf
12. Hamann MT. (2005). Methods in Biotechnology. Natural Products Isolation, Edited by Satyajit D. Sarker, Zahid Latif, and Alexander I. Gray. Humana Press, Totowa, NJ. xii+ 515 pp. 16× 24 cm. ISBN 1-588-29-447-1. $135.00.DOI:10.1021/jm0680068
13. Eroglu P, Alzaim M, Binzet R. (2024). Total phenolic content, phytochemical profile and cytotoxic effects of endemic *Alyssum floribundum* (Brassicaceae) from Turkey. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology. 3;158(4):641-9. DOI:10.1080/11263504.2024.2347855
14. Yugandhar P, Rao KM, Sengupta K. (2018). A novel herbal composition containing extracts of Boswellia serrata gum resin and *Aegle marmelos* fruit alleviates symptoms of asthma in a placebo controlled double-blind clinical study. Phytotherapy Research. ;32(1):140-50. DOI: 10.1002/ptr.5963
15. Adhikari M, Kharel N, Gaire L, Poudel R, Shrestha SM, Gaire SP, Acharya B. (2018). *In vitro* evaluation of different chemicals against Rhizoctonia solani by poisoned food technique. Field Crop.20;1. DOI.10.5376/fc.2018.01.0002
16. Ismail M, Haq N, Ali S, Ali I, Shaheen R, Murtaza G. (2017). Qualitative analysis for phytochemicals of selected medicinal plants from Gilgit-Baltistan, Pakistan. Asian J Chem.29(9):1929-32. DOI:10.14233/ajchem.2017.20583
17. Kumar RS, Venkateshwar C, Samuel G, Rao SG. (2013). Phytochemical Screening of some compounds from plant leaf extracts of *Holoptelea integrifolia* (Planch.) and *Celestrus emarginata* (Grah.) used by Gondu tribes at Adilabad District, Andhra Pradesh, India. International Journal of Engineering Science Invention. 2(8):65-70. https://ijesi.org/ papers/Vol%202(8)/Version-2/L0282065070.pdf
18. Carvajal-Moreno M. (2017). Do gastroenterologists consider aflatoxins as origin of digestive system cancers. Journal of Pharmacovigilance. 5(5).. DOI:10.4172/2329-6887.1000242
19. Abdallah MF, Ameye M, De Saeger S, Audenaert K. (2019). Biological Control of Mycotoxigenic Fungi and Their. Mycotoxins: Impact and management strategies. 59. http://dx.doi.org/10.5772/intechopen.76342.