Int.J.Curr.Microbiol.App.Sci (2026) *15*(2): 228-235

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 2 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1502.023

Antagonistic Activity of *Averrhoa carambola* L. Leaf Endophytes against Plant Pathogenic Fungi

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Keywords

Endophyte, Antagonism, Inhibition, Biocontrol agent

Article Info

Received:
20 December 2025
Accepted:
31 January 2026
Available Online:
10 February 2026

A B S T R A C T

Endophytes are the crucial microorganisms, play a key role as Biocontrol agent. The present paper aims at documenting the effects of Endophytes isolated from Averrhoa carambola L leaf. Three Endophytes were isolated from leaf margins after inoculating it on PDA media plates by Imprint method & identified as *Dematiaceous fungi 1*(E_1), *Dematiaceous fungi* 2(E_2) & *Fusarium species*(E_3) based on morphological & microscopic characters. The potentiality of all three endophytic fungi were checked for its antagonism towards *Didymella bryoniae* (P_1), *Aspergillus species* (P_2), as pathogens respectively by dual culture technique. After an incubation period of 30 days, the Percent inhibition (PI)of pathogen is calculated. The percent inhibition of E_1 against P_1 & P_2 is 40% & 40.6% respectively. E_2 against P_1 & P_2 is 38.6% & 32%, E_3 against P_1 & P_2 is 80.6% & 82.6% respectively. The order of activity is $E_3P_2 > E_3P_1 > E_1P_2 > E_1P_1 > E_2P_1 > E_2P_2$. The research work concludes that Fusarium species as Biocontrol agent is a promising Endophyte that can supress many pathogens particularly *Didymella bryoniae* which causes Gummy stem blight of cucurbits by more than 80%.

Introduction

Micro-organisms play a very important role in the environment. Every activity or process in the environment of biosphere, from the mobilization of elements to till the production of highly valuable commercial antibiotics is governed by micro-organisms. Therefore, it is important to discover the microbes and its applications in the field of agriculture, medicine, industry. One such discovery is class of micro-organisms that has been isolated from plant parts commonly known as 'Endophytes'. The term 'Endophytes' was originally defined by 'De Bary' in (1866) as "Any organism occurring within plant tissues". Endophytes invade the living tissues of plants and they cause asymptomatic infections within the tissues but will cause no symptoms of disease (Wilson, 1995). The definitions for Endophytic micro-organisms which is widely accepted was given by Bacon and White (2000) as "Microbes that colonize living, internal tissues of plants without causing any immediate negative effects".

One more classical definition is "Micro-organisms that colonize plant tissues without producing any apparent symptoms or obvious negative effects". Organisms occupy the intercellular spaces of stems, petioles, roots, and leaves of plants. This Fungi was beneficial to the host which increased the tolerance of biotic and abiotic

stress factors. The discovery led to the search of new Endophyte species.

Various Endophytes were isolated from different plants growing under different environmental conditions that includes tropic, temperate, xerophytic and aquatic environments. Endophytes get transferred from one plant to another plant through seeds. Number of Endophytic populations differ based on the environmental conditions in which the host plant grows. Especially the plant of special ethno-botanical uses having extreme age produce novel Endophytes.

Abundant number of Ascomycetes were found as Endophytes whereas Basidiomycetes, Deuteromycetes and Oomycetes are rarely found. Certain fungal species occur in greater frequency representing particular families and invade host plants. Endophytes are of commercial use, they have pharmaceutically promising characters like antibiotics, chemotherapeutic agents, secondary metabolites etc. They are chemical synthesizers. Secondary metabolites like alkaloids, benzopyramones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, xanthones etc. Endophytes presently gained much importance in agriculture as biocontrol agent to supress the disease causing pathogens. So the need for its isolation and utilisation is increasing day by day. The present work focuses on objectives like isolation, identification and to study potentiality of endophyte as antagonist against pathogens.

Materials and Methods

Study area

Averrhoa carambola L. The plant with much Ethnobotanical importance is collected from the nursery Chandravana near the main campus Manasagangotri, University of Mysore.

Collection of Plant material

Mature healthy plant material of ***Averrhoa carambola.L*** is selected and collected. The plant is identified by the expertise, Department of Botany, University of Mysore. The collected samples are brought to the laboratory immediately and used within 12 hrs of time. The fresh material can be stored in the refrigerator for further use but Long term stored samples cannot be used for the experiment. Plant tissues such as leaves, stem, root, petiole are widely used to isolate the endophytic fungi.

Sample Disinfection

The leaves from which the endophyte to be isolated were washed in running tap water to remove the dust, The fresh leaf samples are cut into bits of 1 cm each and are immersed in 70% ethanol for 1-3minutes and let blot dry, followed by, treating it with 4% aqueous sodium hypochlorite solution for 1 minute & rinsing the sample with distilled water 4-5 times

Isolation of Endophytic fungi by Imprint method

The surface sterilized leaf bits are pressed on the potato dextrose agar media plates which leaves an imprint of surface micro-organism if present. This is referred to as imprint plate, in which abaxial and adaxial imprints are taken. Additional plate called Replica plate or main plate where the imprinted leaf bit is inoculated (fig no 1). The fungi that emerges from the cut margins of leaf are confirmed as endophyte. The obtained endophytic fungi is isolated and sub-cultured on potato dextrose agar media enriched with chloramphenicol to avoid bacterial contamination.

Isolation of pathogenic fungi

The pure culture of pathogen *Didymella bryoniae* is collected from the Department of Botany, Manasagangotri, University of Mysore & *Aspergillus sps* is isolated from infected *Cajanus cajana* seeds.

Antagonistic activity by dual culture method

The main criterion of this assay is to check percent inhibition of pathogens by endophytic fungi. Dual culture method involves the activity of the two fungi, where in the endophytic fungi is placed at one pole 2cm away from the wall of media plate and the pathogen is placed at another pole with a 9cm distance in between. The growth of the fungi is measured in terms of 'CM'. control is used to measure actual growth of Endophyte individually.

Percent inhibition of fungi is calculated by the formula:-

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

Where,

PI = Percent inhibition over control.

C = Growth of test pathogen with absence of antagonist (cm).

T = Growth of test pathogen with antagonistic (cm).

Results and Discussion

Three Endophytic fungi were isolated from the leaves of *Averrhoa carambola.L* & identified them as Dematiaceous fungi (DM1), Dematiaceous fungi (DM2) which shows a characteristic dark brown and Green colour velvety colonies respectively & *Fusarium* sps showing cottony white colony with elongated spores(fig no 2) Here the Endophytes are designated as E1, E2, E3. These endophytic fungi were tested for its potentiality towards antagonistic activity to inhibit the pathogens like *Didymella bryoniae, & Aspergillus sps* by dual culture technique. Here Pathogens are designated as P1, P2. (fig no 3). The morphological characters and microscopic characters of both E's & P's at 10X & 40 X magnification were observed & its pure cultures were maintained on PDA media substituted with chloramphenicol antibiotic. A point inoculation of an Endophyte & a pathogen was made on opposite poles of media plate & kept for an incubation period of 18-30 days at room temperature to check the percent inhibition. The growth of fungi in both dual culture plates and control plates are measured in 'cm' or 'mm' & tabulated. (Table No1 & 1.1).

The first Endophyte Dematiaceous fungi 1 showed the inhibitory activity against pathogen *Didymella bryoniae* by 40% and against pathogen *Aspergillus sps* by 40.6%, at an incubation period of 30 to 34 days(fig no 4) Order of activity is as follows $E_1P_2 > E_1P_1$.

The second Endophyte Dematiaceous fungi 2 showed the inhibitory activity against pathogen *Didymella bryoniae* by 38.6% and against *Aspergillus species* by 32%, at an incubation period of 18 to 30 days. Order of activity is as follows. $E_2P_1 > E_2 P_2$. The Endophyte *Fusarium sps* showed inhibitory activity against *Didymella bryoniae* by 80.6% and against *Aspergillus spp* by 82.6%, at an incubation period of 30 days & its Order of the activity is as follows. $E_3P_2 > E_3P_1$. (see fig no 3)

Complete order of activity: $E_3P_2 > E_3P_1 > E_1P_2 > E_1P_1 > E_2P_1 > E_2P_2$

Sun *et al.,* (2014) have isolated and identified 25 fungal endophytes associated with roots of *Santalum album* and *Kuhnia rosmarianifola.* The isolated endophytes were spp of Pencillium and Fusarium & Tapwal *et al.,* (2016) isolated endophytic fungi from the sterilized leaves, wood, bark and twigs of Sandal wood.

He used different medias like Potato Dextrose Agar, Czopex Dox Agar (CDA), yeast mannitol Agar (YMA) and water agar (WA). The endophytes obtained were identified as *Fusarium oxysporum* (non-pathogenic), *F. solani*, *Histoplasma spp* and *Periconia* spp and *Pestalatiopsis* spp. All the endophytes were checked for their antifungal activity against pathogenic *Fusarium oxysporum*. Maximum inhibition was shown by *Pestalotiopsis spp* (40.40%).

The same antagonistic activity work was done by Sundaramoorthy. S *et al.,* 2013[12] by dual culture method using *Trichoderma spp* against wilt of tomato caused by *Fusarium oxysporum* and *Fusarium lycopersici*. The *F. oxysporum, F. lycopersici* were isolated from infected tissues of stem and root. The percent of inhibition by one of the isolates of trichoderma ANR-1 inhibit the growth of *F.oxysporum*, and *F.lycopersici* by 53.0% followed by KGI-3(38.12%), RTM-5 6(31.11%) and KPI-9(27.2%).

A Comparative account on results of above said works and the present work reveal that *Fusarium sps* as an endophyte, it inhibits the growth of pathogens like *Didymella bryoniae*, *Aspergillus sps* by 80.6 & 82.6% respectively, Whereas the *F. oxysporum* and *F. lycopersici* as pathogens were inhibited by the Trichoderma sps (Sundarmoorthy. S *et al.,* 2013) and as a pathogen it also inhibited the growth of several endophytes (Yadav. R *et al.,* 2015). Fusarium species can be both pathogenic and also bio control fungi.

Fungal endophytes associated with medicinal plants have potential role to promote plant growth. Mutualistic relation between host and endophyte results in benefits of both. Endophytes provide protection to its host by producing various lethal chemicals that can affect only the pathogen.

Table.1 Showing percent inhibition of Pathogens by Endophytes after an incubation period of 30 days

Antagonism

Sl no	Name of Endophyte (E)	Antagonistic activity against Pathogens	Growth of 'E'	C (control pathogen)	T (pathogen against endophyte)	PI in %
1	*Dematiaceous fungi(DM1)*	*Didymella bryoniae*	1.9cm	4.9 cm	3.6 cm	26.5%
		Aspergillus sps	2.0cm	3.5 cm	2.4 cm	31.4%
2	*Dematiaceous fungi(DM1)*	*Didymella bryoniae*	1.3cm	4.5 cm	3.0 cm	33.3 %
		Aspergillus sps	1.4cm	3.6 cm	2.6 cm	27.7%
3	*Fusarium sps*	*Didymella bryoniae*	6.4cm	4.9 cm	2.5 cm	55.1 %
		Aspergillus sps	7.4cm	3.5 cm	1.5 cm	57.1 %

Table.2 Showing percent inhibition of Pathogens by Endophytes after an incubation period of 18 days

Sl no	Name of Endophyte(E)	Antagonistic activity against Pathogens	Growth of 'E'	C (control pathogen)	T (pathogen against endophyte)	PI in %
1	*Dematiaceous fungi(DM1)*	*Didymella bryoniae*	5.9cm	8.5 cm	5.1 cm	40%
		Aspergillus sps	2.6cm	5.9 cm	3.5 cm	40.6%
2	*Dematiaceous fungi(DM1)*	*Didymella bryoniae*	2.5cm	7.5cm	4.6 cm	38. 6 %
		Aspergillus sps	2.3cm	5.3cm	3.6 cm	32.0%
3	*Fusarium sps*	*Didymella bryoniae*	6.5cm	6.2cm	1.2cm	80.6%
		Aspergillus sps	7.8cm	6.9cm	1.2cm	82.6%

Fig.1 Diagrammatic representation of imprint method. A. Main plate, B. imprint plate

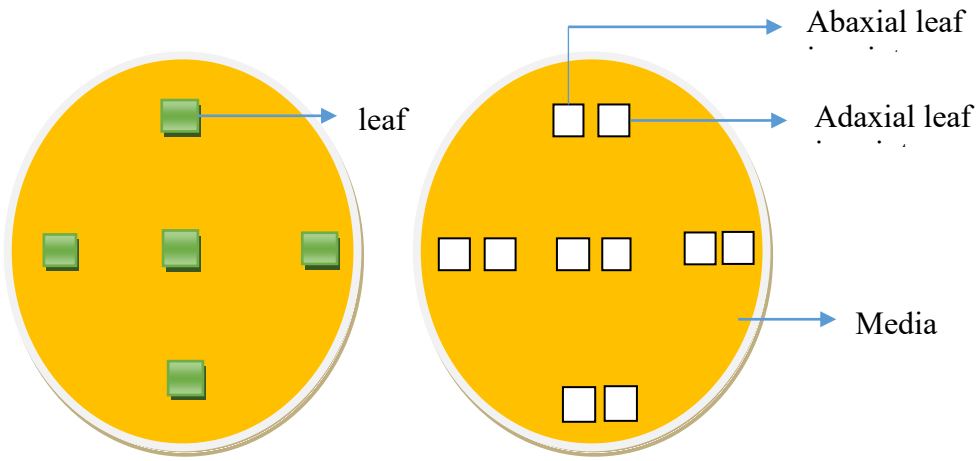


Fig.2 Endophytes E1 is Dematiaceous fungi colony, (E1-a) is Microscopic view of mycelia under 10X, (E1-b) Microscopic view of mycelia under 40X. Endophyte 2; Dematiaceous fungi 2, (E2-a) microscopic view of mycelia under 10 X, (E2-b) microscopic views of mycelia under 40X. Endophyte E3 Fusarium sps, (E3-a) microscopic view of mycelia under 10X, (E3-b) microscopic view of mycelia under 40X.

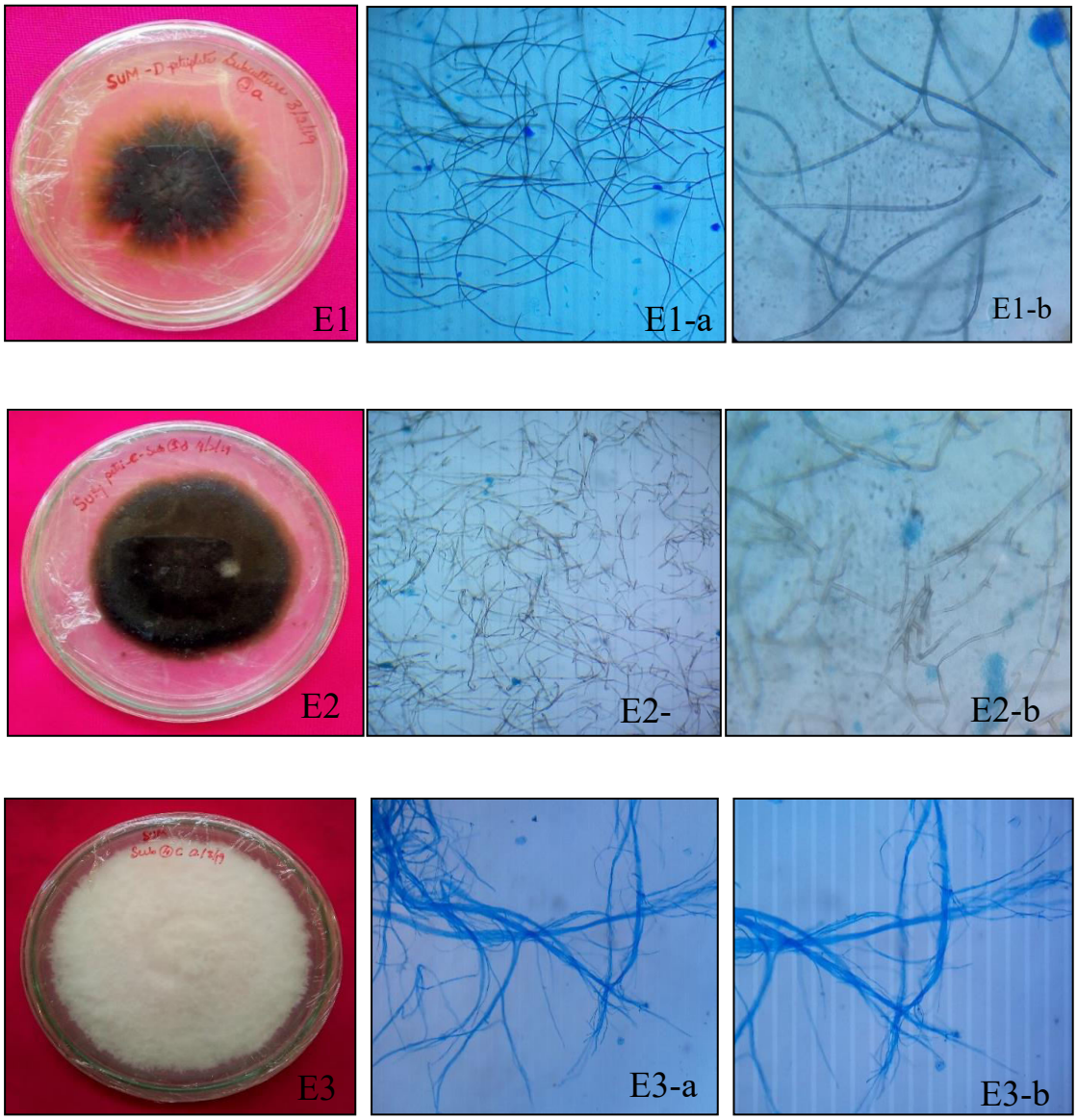


Fig.3 Pathogens; P1 is *Didymella bryoniae,* (P1-a) microscopic view of mycelia under 10X, (P1-b) microscopic view of spores under 40X. P2 is pathogen Aspergillus species, (P2-a) microscopic view of mycelia and conidia under 10X, (P2-b) microscopic view of spores under 40X

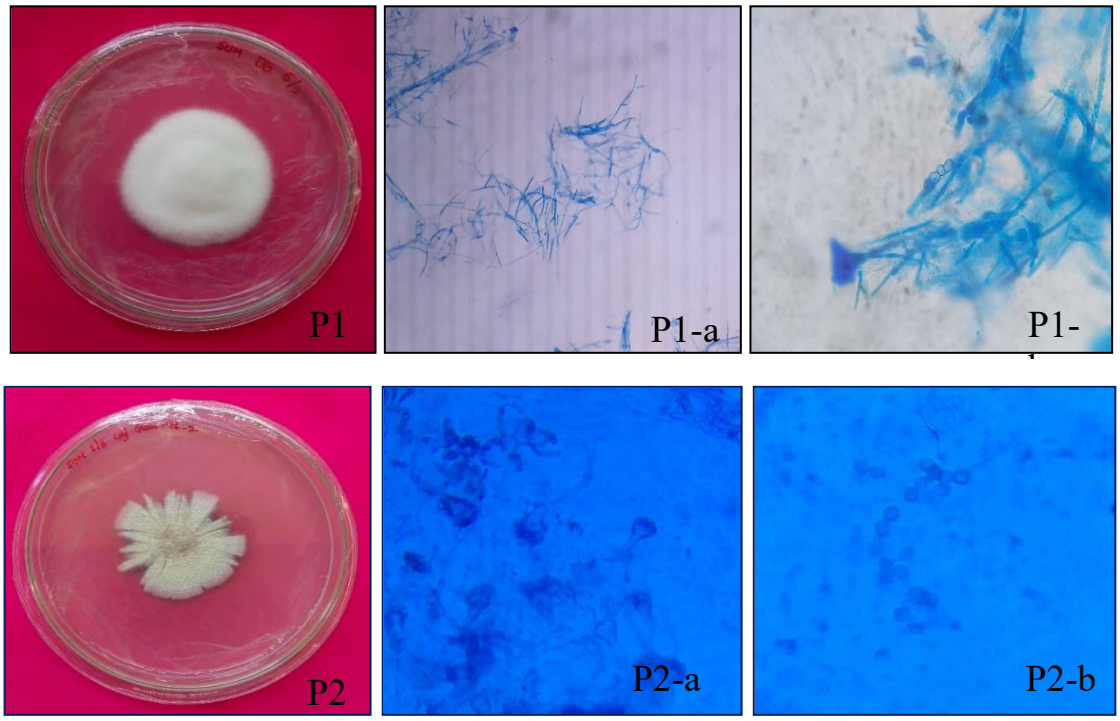
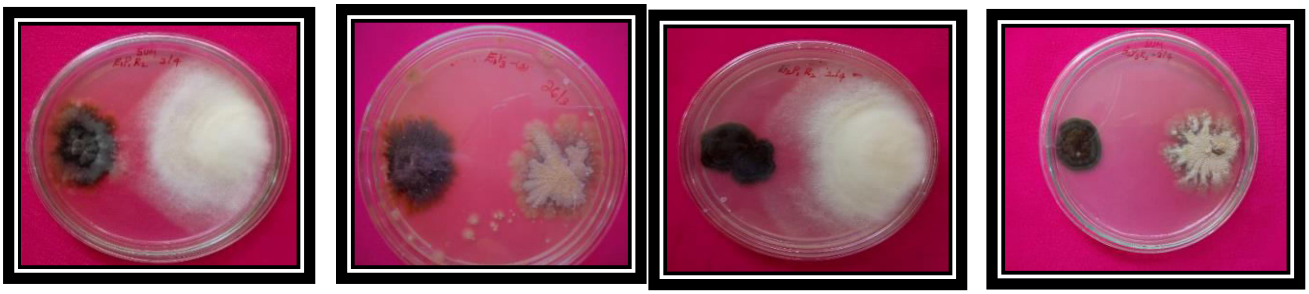


Fig.4 Showing Antagonistic activity of Endophytes against Pathogens (E_1, E_2, E_3 Vs P_1, P_2)



E_1 Vs P_1 E_1 Vs P_2 E_2 Vs P_1 E_2 Vs P_2

E_3 Vs P_1 E_3 Vs P_2

As Endophytes have potential to inhibit the pathogens, it can be used as biocontrol agent to cure the plant disease caused by various fungi. *Fusarium* sps isolated from *Averrhoa carambola*.L leaves can be used to reduce the

"Gummy stem blight" in cucurbits such as watermelon & muskmelon caused by *Didymella bryoniae* by more than 80%.

In conclusion, Examination of Antagonistic activity of Endophytes against Pathogens revealed its potentiality to supress/inhibit the disease-causing fungi of plants. Its activity has shown promising effects in laboratory, which can be used as best biocontrol agent in agriculture to prevent crop loss. Fusarium sps particularly shown more potential in inhibiting the *Didymella bryoniae* by 80.6% and Aspergillus sps by 82.6%. Fusarium as a biocontrol agent shows much similarity to Trichoderma.

Acknowledgement

The Author is thankful to the Head, Department of studies in Botany, Manasa Gangotri, University of Mysore for providing the necessary isolates and laboratory facilities which has contributed its best of use to complete my research work.

Author Contributions

Maram Sushmitha Shetty: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Bacon, C.W. and White, J.F. (2000). Microbial endophytes. Marcel Dekker, New York, pp 341-388.

Bhagya, B. and Manjula, S. (2013). Morphological and Biochemical Characteristics of Averrhoa fruits. *International journal of Pharmaceutical, Chemical and Biological sciences*. 3(3): 924-928.

Chowdhury, I.H. and Sourav, G. (2014). Green synthesis of water dispersible silver nanoparticles at room temperature using green carambola (star fruit). *J sol-Gel sci technol*.

Das, B.N. and Ahmed, M. (2012). Analgesic activity of the fruit extract of *Averrhoa carambola. International journal of life sciences Biotechnology and Pharma Research.* Vol 1, No3.

Grazia Campanile, Angela Ruscelli, Nicola Luisi (2007). Antagonistic activity of endophytic fungi towards *Diplodia corticola* assessed by in vitro and in planta tests, Volume 117, pages 237–246.

Gunwardena, D.C., Jayasinghe, L and Fnjimoto Y. (2015). Phytotoxic constituents of the fruits of *Averrhoa carambola*. *Chemistry of Natural Compounds*, Vol 51, No.3: 532-533.

Hanqiao Liang, Yongmei Xiag, Juan Chen, Dawei Zhang, Shunxing Guo, Chumian Wang. Antimicrobial activities of Endophytic fungi Isolated from Ophiopogon Japonicus

Hansraj M., Vyas, K., Pandya, A and Singhal, G (2012). A complete review on *Averrhoa carambola. World journal of Pharmacy and Pharmaceutical sciences*. Vol 1:17-33.

Hyde, K.D. and Soytong, K. (2008). The Fungal endophyte dilemma. *Fungal diversity* 33: 163-173.

K. Talapatra., A. Roy Das., A. K. Saha, P. Das (March-April, 2017). In vitro antagonistic activity of a root endophytic fungus towards plant pathogenic fungi, Journal of Applied Biology & Biotechnology Vol. 5 (02), pp. 068-071.

Kaul, S., Gupta, S., Ahmed, M., and Dhar M.K. (2012). Endophytic fungi from Medicinal plants: a treasure hunt for bioactive metabolites. *Phytochemistry Reviews*, 11(4): 487-505.

Manjari, M.P., Santosh, K. S., Gautam, K.N. and kulamani. R (2015). Biomimetic synthesis, Characterization and mechanism of formation of stable silver nanoparticle using *Averrhoa carambola* leaf extract. *Materials letters*, 160: 566-571

Martínez-Álvarez P, Alves-Santos FM, Diez JJ. In vitro and in vivo interactions between Trichoderma viride and Fusarium circinatum. Silva Fennica. 2012; 46(3): 303–316

Masroor quadri, Sarojini Johri, Bhahwal A shah, Anamika Khajuria, Tabasum sidiq, Surrinder k lattoo, Malik Z Abdin & Syed Riyaz-UI- Hassan

2013. Identification and bioactive potential of endophytic fungi isolated from selected plants of the western Himalayas. A springer open Journal.

Naik BS, Shashikala J, Krishnamurthy YL, 2009. Study on the diversity of endophytic communities from rice (Oryza sativa L.) and their antagonistic activities in vitro. Microbiological Research; 164:290-296

Nashwa Sallam., Esmat F. Ali., Mohamed A., A. Seleim.,Hadeel M. M. Khalil Bagy, (2021). Endophytic fungi associated with soybean plants and their antagonistic activity against *Rhizoctonia solani,* Volume 31, article number 54,

Owen, N.L. and Hundley, N. (2004). Endophytes-the chemical synthesizers inside plants. *Science Progresses* 87(2): 79-99.

Pannida Khunnamwong, Noppon Lertwattanasakul, Sasitorn Jindamorakot, Nakarin Suwannarach, Kenji Matsui, Savitree Limtong, (2020). Evaluation of antagonistic activity and mechanisms of endophytic yeasts against pathogenic fungi causing economic crop diseases, Volume 65, pages 573–590

Rahul, Y., Ajay, V., Samiksha, J and Manish, K (2015). Antifungal and enzyme activity of endophytic fungi isolated from *Oscimum sanctum and Aloe vera*. *African Journal of Microbiology Research*. Vol 9(29). Pp 1783-1788.

Ruby, E.J. (2013). Materials and Methods. *Bioprospecting for microbial endophytes and their natural products*: 47-93.

Si-sheng SUN, Xiao-mei CHEN, Shun-xing Guo (2014). Analysis of endophytic fungi in roots of *Santalum album* Linn. & its host plant *Kuhnia rosmarinifolia* Vent. Jounal of Zhejiang university-SCIENCE B.

Soncini, R., Santiago, M.B., Orlandi, L., Moraes, G.O.I., Peleso, A.L.M., Santos, M.H.D., Alves-da-silva, G., Paffaro, V.A., Bento, C.A and Alexander, G.P (2011). Hypotensive effect of aqueous extract of *Averrhoa carambola* (oxalidaceae) in rats: An invivo and in vitro approach. *Journal of Ethnopharmacology* 133: 353-357.

Sundaramoorthy, S. and Balabaskar.P (2013).Biocontrol efficacy of Trichoderma species against wilt of tomato caused by *Fusarium oxysporum* f. sp. lycopersici. *Journal of Applied Biology and Biotechnology* vol 1(03): 36-40.

Svetlana, Z.S., Stojanovie, Z., Anovic, Jelica B (2010). Screening of antagonistic activity of micro-organisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporoides*. *Arch. Boil. Sci. Belgrade* 62: 611-623

Terhonen E, Kerio S, Sun H, Asiegbu FO. Endophytic fungi of Norway spruce roots in boreal pristine mire, drained peatland and mineral soil and their inhibitory effect on Heterobasidion parviporum in vitro. Fungal ecology. 2014; 9:17-26.

Thomas, S., Patil, D.A. and Naresh Chandra. (2008). Pharmacognostic evaluation and Physicochemical Analysis of *Averrhoa carambola* fruit. *Journal of Herbal Medicines and Toxicology* 2(2): 51-54.

Valsan, A. and Raphael, R.K. (2016). Pharmacognostic profile of *Averrhoa bilimbi* Linn leaves. *South Indian Journal of Biological Sciences*, 2(1): 75-80.

Wilson, D. (1995) Endophytes—the evolution of a term and clarification of its use and definition. Oikos 73, 274–276.

Zeng wei lu, hao yang liu, Chen-lu Wang, Xue Chen, Yi-Xiao, huang, Miao-Miao Zhang, Qui-Liang Haung and Guo- Fang Zhang, *2023*. Isolation of endophytic fungi from cotoeaster multiflorus and screening of drought-tolerant fungi and Evaluation of their growth promoting Effects. *Frontiers in Microbiology.*

Zhang, H. W., Song, Y. C. and tan, R.X. (2006). Biology and chemistry of endophytes. *Natural Product Reports*, 23: 753-77.

Zoha Daroodi, Parissa Taheri, Saeed Tarighi, Direct antagonistic activity and tomato resistance induction of the endophytic fungus *Acrophialophora jodhpurensis* against *Rhizoctonia solani,* volume 160, September 2021, 104696.

How to cite this article:

Maram Sushmitha Shetty. 2026. Antagonistic Activity of *Averrhoa carambola* L. Leaf Endophytes against Plant Pathogenic Fungi. *Int.J.Curr.Microbiol.App.Sci.* 15(2): 228-235. **doi:** https://doi.org/10.20546/ijcmas.2026.1502.023