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## Plant extraction mediated mitigation of chilli fruit rot caused by *Colletotrichum* spp.

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**Abstract**

Six isolates of *Colletotrichum* spp. isolated from the fruit rot infected samples collected from various chilli growing areas of Tirunelveli and Thoothukudi districts. Among the six isolates of *Colletotrichum* spp. isolate collected from Ambasamudram (ASD) was most virulent. To manage the chilli fruit rot pathogen *Colletotrichum* sp. (ASD), nine plants extracts viz., *Lawsonia inermis*, *Azadirachta indica*, *Bougainvillea spectabilis*, *Withania somnifera*, *Ocimum tenuiflorum*, *Aegle marmelos* L., *Justicia adhatoda*, and *Calotropis gigantea* were tested under *in vitro* condition through poisoned food technique. Among them ten per cent leaf extracts, of *Withania somnifera* (10%) highly inhibit the mycelial growth of *Colletotrichum* SP (ASD) (84.88 per cent) followed by *Aegle marmelos* L., (85 per cent) and *Azadirachta indica* (81.10 per cent reduction over control).

**Keywords:** Anthracnose, *Colletotrichum* spp, plant extract, chilli fruit rot

**Introduction**

Chilli (*Capsicum annum* L.) is a spice crop being cultivated all around the world occupying very important role in human daily diet. Its belongs to the solanaceae family. It is cultivated mainly under tropical and sub tropical climatic conditions. Chilli is highly rich in vitamin A, B, C (Ascorbic acid) and E (Tocopheral), oleoresin, carbohydrates and minerals such as calcium, phosphorus, ferrous, sodium and copper in trace amounts (Prathibha *et al*, 2013). Chilli cultivation is mainly affected by fungal, bacterial and viral disease. Among them chilli fruit rot caused by *Colletotrichum* spp. is highly devastating one leads to yield reduction and less marketable value. Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu are the major chilli growing states in India which together contribute about 75 per cent of the total cultivated area (Rajesh Kumar *et al.*, 2011). The chilli fruit rot disease has been reported to cause 30-76 per cent yield loss in Tamil Nadu (Datar, 1995). Recent survey revealed that *C. capsici* is the most predominant species in the major chilli growing states namely Karanataka, Andhra Pradesh and Tamil Nadu in India (Ramachandran *et al.*, 2008). Nowadays numerous synthetic fungicides are used against plant disease and this resulted in polluted environment harmful to the livelyhood animals and human. Application of over dosage of chemicals attempt to overcome anthracnose disease enhance the residual toxicity level in chilli fruits after harvest. In current scenario of Indian agriculture plants disease control methods involving biocontrol agents and botanicals are highly needed to protect the soil and crop ecosystem. Use of botanicals for the management of the chilli fruit rot is very cheap and also environmentally safe.

**Materials and Methods****Collection of samples and isolation of pathogen**

Chilli fruit rot infected samples were collected from various places of Tirunelveli districts namely viz., Ambasamudram, Kaluneer Kulam, Kzheel Surandai, and Surandai also from Killikulam, Kovil Patty of Thoothukudi districts and preserved for pathogen isolation.

**Table 1:** isolation name and place of collection of chilli fruit rot infected samples

S. No	Districts	Village	Isolate Name
1	Tirunelveli	Ambasamudram	ASD
2	Tirunelveli	Kaluneer Kulam	KNK
3	Tirunelveli	Kzhell Surandai	KS
4	Tirunelveli	Surandai	S
5	Thoothukudi	Killikulam	KKM
6	Thoothukudi	KovilPatty	KPT

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**Isolation of *Colletotrichum* spp.**

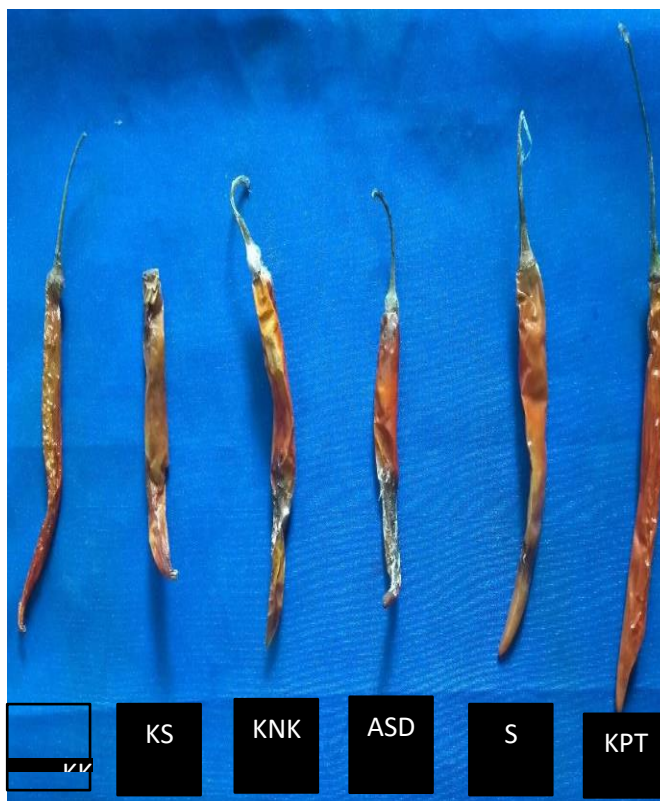
Infected fruit sample collect from various place of Tirunelveli and Thoothukudi district. The infected tissue was cut into small piece and surface sterilization with 0.1% sodium hypochloride in 30 sec followed by washing with distilled water for 2-3 times. The sterilized PDA medium was poured in sterilized Petri Plates and allowed to solidify fruit samples were placed on the center of plates incubated at 25±3oc for 10 days.

Morphological characters of the isolates Ten mm culture disc of seven days old pathogen was cut using a sterilized cork borer and placed at the center of each sterile petri plate containing 20ml of PDA medium. The plate was incubated at room temperature (28±20c) for 10 days. The growth and morphological characters of the isolates viz., colour of mycelium and shape and colour of the conidia were observed. Among the six isolates ASD isolate was fast growing and more virulent than other isolates. The colour of mycelium grey, shape of conidia is sickle shape after 20th days of inculation the old culture started producing acurvuli. Hence, ASD isolate was taken for further studies.

**Pathogenicity test**

Six *Colletotrichum* isolates were seperately taken from well grown PDA in fourteen days old cultures and maintain separately. The conidial suspension of above isolates was prepared. The petriplates were flooded with sterilized distilled

water and gradually scrapped by using sterilized loop. Conidia were collected from petriplates individually and spore suspension was filtered through sterilized muslin cloth. The conidial suspension was adjusted to 106 conidia ml- 1 using haemocytometer. Then the pre matured and matured chilli fruits were collected. These fruits were surface sterilised with 1% sodium hypochlorite solution for 5 minutes and finally two to three times rinsed with distilled water. The conidial suspension of six isolates were individually collected in sterilized tubes. Then each conidial suspension of 10 microlite was injected on the sterilized fruit surface with using sterilized syringe. Three replications were taken maintained for each isolates kept in moist chambers at 25oc. Then inoculated fruits were evaluvated after 10 days. Anthrnose symptoms severity based on the size of the disease severity level. The disease severity was scored on a 0-9 scale given by Montri *et al.* (2009). The grade was indicated as follow 0-grade =no infection on fruit, 1grade =1- 2%, 3grade =3-5%, 5grade =6-10%, 7grade =11-25% and 9grade =>25% infected fruit area. The pathogen was reisolated from the fruit showing symptom and it was compared the original culture. The comparision done to prove the Koch's postulates. In this study, all the six isolate resembles the original isolate. Among the various isolates the isolate ASD showed maximum lesion index in pathogenicity study (>25%) so its forward for testing efficacy of botanicals.



**Fig 1:** Proving pathogenicity of various isolates

**Table 2:** Various isolates disease severity level

Score	Disease severity level						severity
Isolates	ASD	KKM	KS	KNK	S	KPT	
0	-	-	-	-	-	-	No infection
1	-	-	-	-	-	-	Larger necrotic lesions
3	-	>2-5%	-	-	>2-5%	-	Water soaked lesions on fruit surface
5	-	-	-	>5-15%	-	-	Necrotic lesion ans Acervuli present
7	-	-	>15-25	-	-	>15-25	Necrotic lesions and acervuli

**In vitro efficacy of plant extracts against the *Colletotrichum sp.* (ASD)**

Nine medicinal plants viz., Villvam, Henna, Neem, Bougainvillea, Sangupushpam, Adadhoda, Ashwagandha, Eruku, and Thulasi were collected and its efficacy against *Colletotrichum* SP was tested through poisoned food technique.

**Table 3:** List of botanicals tested against *Colletotrichum* spp.

S.no.	Scientific name	Common name	Parts used
1	<i>Lawsonia inermis</i>	Henna	Leaf
2	<i>Azadirachta indica</i>	Neem	Leaf
3	<i>Bougainvillea spectabilis</i>	Bougainvillea	Leaf
4	<i>Withania somnifera</i>	Ashwagandha	Leaf
5	<i>Ocimum tenuiflorum</i>	Thulasi	Leaf
6	<i>Aegle marmelos L.</i>	Villvam	Leaf
7	<i>Justicia adhatoda</i>	Adhatoda	Leaf
8	<i>Calotropis gigantean</i>	Eruku	Leaf
9	<i>Clitoria ternatea</i>	Sangupushpam	Leaf

One gram leaf samples of above mentioned botanicals taken. They were ground in fine paste with one ml of sterile water with help of pestle and mortar. Initially the extract was filtered through the cotton muslin cloth and finally they were filtered through the bacterial proof filter to avoid bacterial contamination. This formed a standard plant extract solution

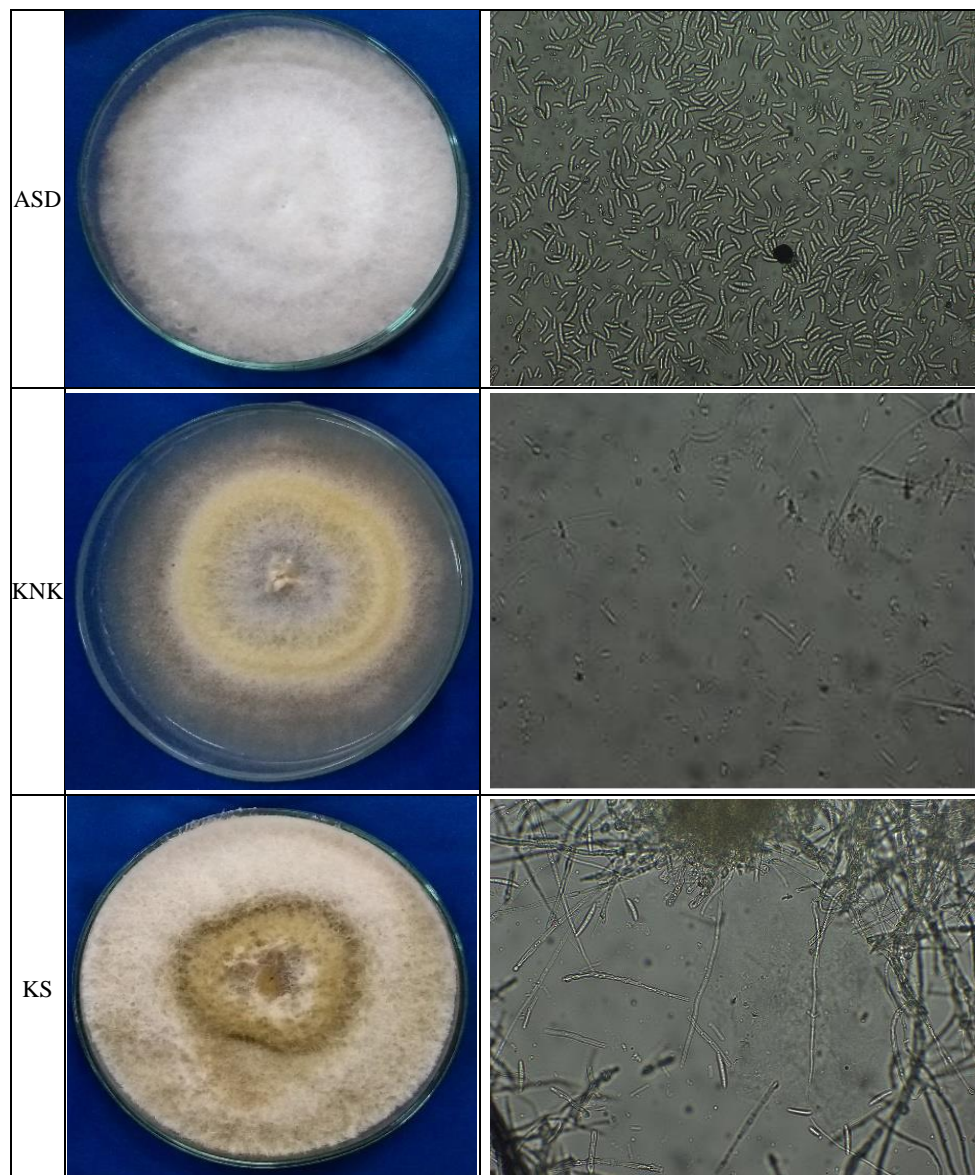
(100%). The extract was further diluted into 5 and 10 percent concentration using sterile PDA medium.

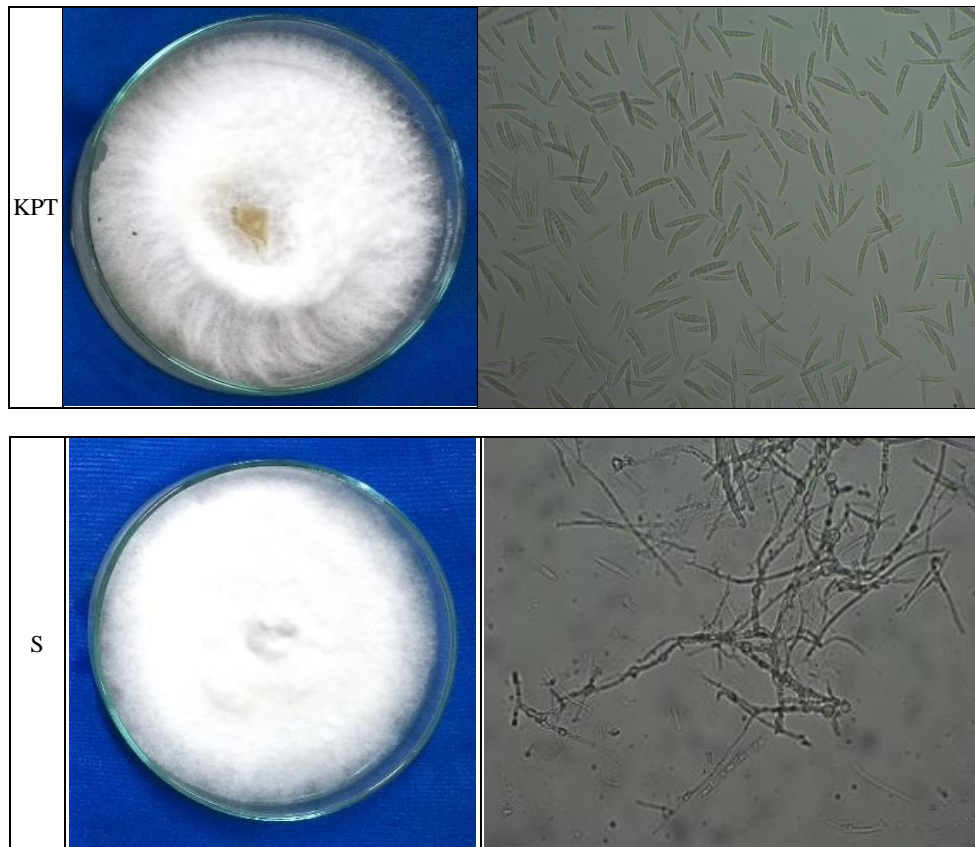
**Effect of plant extract on the growth of *Colletotrichum sp.* (ASD)**

The plant extract solution was mixed with PDA medium to obtain 10 percent concentration. A nine mm actively growing PDA culture disc of *Colletotrichum sp* was cut by sterilized Cork borer and placed at the center of the medium. The plates were incubated at room temperature (28±20c). PDA without Plant extract served as control. Three replications were maintained for individual treatment. The radial growth of the mycelium was measured in treatment on 10th day after inoculation when the fungus was fully grown (9cm) in the control plate. The mean diameter of the mycelial growth of the pathogen was recorded and the result was expressed in terms of percent inhibition of mycelium over control.

**Result and discussion****Isolation and proving the pathogenicity of chilli fruit rot pathogen *Colletotrichum* spp.**

Six isolates of *Colletotrichum* spp viz., ASD, KKM, KPT, KNK, KS, S are isolated from samples collected from various places of Tirunelveli and Thoothukudi Districts. The growth and morphological characters of all the six isolates were appended in fig 2.



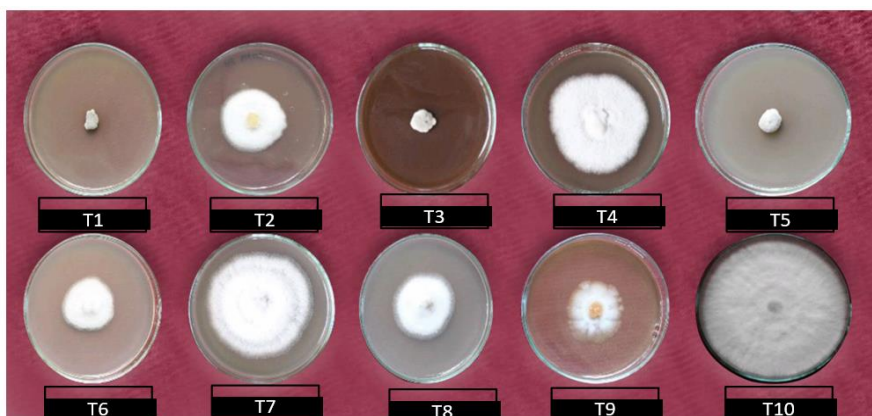


**Fig 2:** The growth and morphological characters of all the six isolates were appended in fig 2.

Among the six isolates ASD isolate was fast growing. Five isolates produce falcate shape conidia except S producing cylindrical shape. Among the six isolate ASD isolate caused high level of infection. Effect of plant extracts on mycelial growth of *Colletotrichum* sp. (ASD) The inhibitory effect of nine different plant extracts viz., Villvam, Henna, Neem, Bougainvillea, Sangupushpam, Adadhoda, Ashwagandha, Eruku, and Thulasi are tested against ASD isolate of *Colletotrichum* spp through poisoned food technique. Among the nine plant extracts, *Aegle marmelos L.*, and *Withania somifera* are highly inhibiting the mycelial growth of *Colletotrichum* sp. (ASD) (85.50 and 84.88 per cent reduction over control respectively) followed by *Azadirachta indica* inhibiting the mycelial growth of *Colletotrichum* spp

(81.10%) over control and the results were appended in table 3.

Rahmam *et al.*, 2011 report that various plant extracts were on the conidial germination of *Colletotrichum capsici* they added that leaf extract of *Azadirachta indica*, showed maximum reduction of conidial germination followed by *Curcuma longa* (rhizome) and *Ocimum sanctum* (leaf). And also Alam *et al.* (2002) tested the effect of ten plant extracts on conidial germination of *Colletotrichum gloeosporioides* and recorded that *Tagetes erecta* (leaf) and *Azadirachta indica* (bark) extracts were most effective in inhibition of conidial germination at 5:1.5 (w/v) concentration. Marinus Ngullie *et al.*, reported that the plant extracts, *Allium sativum* (10%) and *Azadirachta indica* (10%) showed the highest inhibition of mycelial growth of *C. gloeosporioides*. Fig3



- T1- Ashwagandha (*withania somnifera*)
- T2- Thulasi (*Ocimum sp*)
- T3- Villvam (*Aegle marmelos L.*)
- T4- Bougainvillea (*Bougainvillea spectabilis*)
- T5- Neem (*Azadirachta indica*)
- T6- Adhatoda (*Justicia adhatoda*)
- T7- Eruku (*Calotropis gigantean*)
- T8- Sangupushpam (*Clitoria ternatea*)
- T9- Hennaleaf (*Lawsonia inermis*)
- T10- control

**Fig 3:** Showed the highest inhibition of mycelial growth of *C. gloeosporioides*.

### Conclusion

Among the different plant extracts tested against *Colletotrichum* sp (ASD), leaf extract of (10%) *Withania somifera* and *Aegle marmelos* L., Onpar each other inhibiting the mycelial growth of *Colletotrichum* sp (84.88 and 85.50 per cent reduction over control respectively ) and leaf extract of *Clitoria ternatea* leaf extract showed the least inhibition (35.50 per cent reduction over control ).

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