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# *In vitro* evaluation of various botanicals against *Cercospora malayensis* causing leaf spot of okra

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**Abstract**
Okra (*Abelmoschus esculentus* L.) is one of the foremost vegetable crop grown extensively in south Gujarat during *Kharif* as well as summer seasons. *Cercospora* leaf spot incited by *Cercospora malayensis* (Stev. and Solh.) is a minor disease which was less significant earlier but is now considered as one of the emerging disease in south Gujarat region. Out of 8 botanicals tested against *C. malayensis*, cent per cent inhibition of mycelial growth over control was recorded in garlic clove extracts at 10 per cent concentration which was followed by turmeric rhizome extracts (25.19 and 30.52%) at 5 and 10% concentrations.

**Keywords:** Botanicals, *Cercospora malayensis*, poisoned food technique

## Introduction
Okra (*Abelmoschus esculentus* L.) is a nutritious and delicious annual vegetable crop grown in the tropical and sub-tropical regions. It belongs to the family Malvaceae. There are about eight *Abelmoschus* species available in India. Out of these, *A. esculentus* is the only known cultivated species.

In India, total grown in an area of 511 hectares having total production of 6219 million tons with the productivity of 12.0 million tons per hectare (Anon., 2019a) [1]. Andhra Pradesh is the leading producer followed by West Bengal. In Gujarat total area under okra is 36740 hectares and the total production 480690 million tons with the productivity of 11.6 million tons per hectare (Anon., 2019b) [2].

In Gujarat, it is mainly grown in the districts of Vadodara, Surat, Junagadh, Bhavnagar, Valsad, Gandhinagar, Anand and Navsari. The okra crop occupies an area of 6500 hectares, having production 82225 million tons in Navsari district. (Anon., 2019b) [2].

In India, two species of *Cercospora viz.*, *Cercospora malayensis* Stev. And Solh. And *C. abelmoschi* EII. And Ev. Were found to be the cause of leaf spots in okra (Sridharan and Rangaswamy, 1968) [8]. These species differ in production of symptoms. In India, mainly two species of *Cercospora* produce leaf spots in okra *C. malayensis* causes brown, irregular spots and *C. abelmoschi* causes sooty black, angular spots. The affected leaves roll, wilt and fall. The leaf spots cause severe defoliation and are common during humid seasons.

The specific fungal diseases of okra are, *Cercospora* leaf spot caused by *Cercospora abelmoschi* and *C. malayensis*, Damping-off caused by *Pythium* sp. and *Rhizoctonia solani*, Powdery mildew caused by *Erysiphe cichoracearum*, Southern blight caused by *Sclerotium rolfsiii*, Wilt disease caused by *Verticillium alboatrum*, wet rot caused by *Choanephora cucurbitarum* (Raid and Palmateer, 2006) [7] and Alternaria leaf spot caused by *Alternaria chlamydospora* (Atia and Tohamy, 2004) [3].

During the rainy season, sooty leaf spot disease of okra caused by *Cercospora ablemoschi* (Ellis and Everh.) and *C. malayensis* (Stev. and Solh.) is now becoming one of the most important diseases of okra. Now a days, this disease incited by *C. malyensis* and *C. abelmoschi* becomes more severe in southern transition zone. The disease progress upward from lower and upper leaves and infects stem and fruits and produces similar symptoms (Dhancholia and Singh, 1992) [5].

## Materials and Methods
The experiment was carried out in the laboratory of Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari (Gujarat) during 2018-19. The trial was laid out in completely randomized design under laboratory condition.

The present investigation was carried out to evaluate different plant species for the possible presence of fungal toxicant properties against *C. malayensis* by poisoned food technique.

**Plant extracts preparation**

Eight locally available plants *viz*., *Tridax Procumbens* (Pardeshi bhangaro), Nilgiri (*Eucalyptus globulus* L.), *Adathoda vasica* L. (Ardusi), *Duranta repens* L. (Damyanti), Neem (*Azadirachta indica* L.), Castor (*Ricinus communis* L.), Garlic (*Allium sativum* L.) and Turmeric (*Curcuma longa* L.) were used for their antifungal activities against *C. malayensis* following the procedure given by Bambode and Shukla (1973) [4]. Non-infected leaves, bulb and rhizomes of the plants were collected from the local area from Navsari Agricultural University, Navsari. Fresh leaves, bulb and rhizome of respective plants were first washed with tap water and then with sterilized water. Each sample was then homogenized in sterilized distilled water at the rate of 1ml/g of tissues (1:1 V/W) with a homogenizer and filtered through a fine muslin cloth. The filtrate was centrifuged at 5000 rpm for 20 minutes and the supernatant was filtered with sterilized sintered funnel (pore size 1-2 microns), which formed the standard plant extract solution (100%). The extracts were individually incorporated into V8JA medium 5 and 10 per cent concentration in 250ml conical flasks and sterilized at 1.038 kg/cm$^2$ for 15 minutes. These were poured into 90mm sterilized Petri plates with three repetitions for each extract with different concentrations. Control was maintained without extracts for each concentration. All the Petri plates were inoculated with five mm disc of mycelium of the pathogen and incubated at 28 ± 1°C. Five days after inoculation, the radial growth of mycelium was recorded and Per cent inhibition of the test fungus by the botanicals over untreated control was calculated by applying following formula:

The per cent inhibition of pathogen species was calculated as suggested by Vincent (1927) [9].

$$PGI = \frac{DC-DT}{DC} \times 100$$

Where,

PGI = Per cent growth inhibition

DC = Average Diameter of mycelial colony in control treatment (mm)

DT = Average Diameter of mycelial colony in treated set (mm)

**Results and Discussion**

To ascertain the role of plant extract against *C. malayensis* mycelial growth an experiment was conducted with 8 plant extracts. Different concentrations (5 and 10%) of plant extracts (*Tridax procumbens*, Nilgiri, *Adathoda vasica*, *Duranta repens*, Neem, Castor, garlic and turmeric) were tested against pathogen to determine their antifungal activity in *in vitro* (Plate 1 A and 1 B).

The plant extracts were inhibited growth of fungus with increasing concentration in the medium. The botanicals were higher in inhibiting the mycelial growth of *C. malayensis* at 10 per cent and found significantly superior over the other concentrations. The results presented in table 1 revealed that maximum growth inhibition of *C. malayensis* was recorded in garlic clove extracts (75.95 and 100%) which was followed by turmeric rhizome extracts (25.19 and 30.52%) at 5 and 10% concentrations, respectively. Five and ten per cent concentrations leaf extracts of *Tridax Procumbens* (3.04 and 4.95%), Nilgiri leaf extracts (7.62 and 9.53%), *Adathoda vasica* leaf extracts (7.24 and 9.53%), *Duranta repens* leaf extracts (4.58 and 6.78%), Neem leaf extracts (9.53 and 10.30%) and Castor leaf extracts (3.81 and 6.10%) were recorded poor per cent growth inhibition, respectively. Except for garlic clove extracts, all the plant extracts performed moderately to the poor at both concentrations. However, *C. malayensis* was significantly inhibited by garlic extract at 10 per cent concentration.

Similar results were also obtained by Hemachandra *et al*. (2011) [6] evaluated efficacy of 10 per cent water extracts of seven plant extracts for their antifungal activity *in vitro* against *C. beticola* and observed cent per cent inhibition of mycelial growth by extract of *Allium* sp., followed by *Prosopis julifera* and *Datura metel* recording 50.34 per cent and 31.66 per cent reduction over control, respectively.

**Table 1:** Evaluation of botanicals against leaf spot of okra caused by *C. malayensis*

| Sr. No. | Botanicals | Plant part used | Conc. (%) | Average mycelial growth (mm) | Per cent growth inhibition |
|---|---|---|---|---|---|
| 1 | *Tridax procumbens* (Pardeshi bhangaro) | Leaf | 5 | (84.67)* 9.22** | 3.04 |
| | | | 10 | (83.00) 9.13 | 4.95 |
| 2 | Nilgiri (*Eucalyptus globulus* L.) | Leaf | 5 | (80.67) 9.00 | 7.62 |
| | | | 10 | (79.00) 8.91 | 9.53 |
| 3 | *Adathoda vasica* (Ardusi) | Leaf | 5 | (81.00) 9.02 | 7.24 |
| | | | 10 | (79.00) 8.91 | 9.53 |
| 4 | *Duranta repens* (Damyanti) | Leaf | 5 | (83.33) 9.15 | 4.58 |
| | | | 10 | (81.33) 9.04 | 6.78 |
| 5 | Neem (*Azadirachta indica*) | Leaf | 5 | (79.00) 8.91 | 9.53 |
| | | | 10 | (78.33) 8.87 | 10.30 |
| 6 | Castor (*Ricinus communis*) | Leaf | 5 | (84.00) 9.19 | 3.81 |
| | | | 10 | (82.00) 9.08 | 6.10 |
| 7 | Garlic (*Allium sativum*) | Clove | 5 | (21.00) 4.63 | 75.95 |
| | | | 10 | (00.00) 0.70 | 100 |
| 8 | Turmeric (*Curcuma longa*) | Rhizome | 5 | (65.33) 8.11 | 25.19 |
| | | | 10 | (60.67) 7.82 | 30.52 |
| 9 | Control (Test pathogen only) | | | (87.33) 9.37 | 00.00 |
| S Em± | | | | 0.09 | |
| CD at 5% | | | | 0.28 | |
| CV (%) | | | | 2.07 | |

*Figures in parentheses are original values

**Figures outside parenthesis are square root transformed values

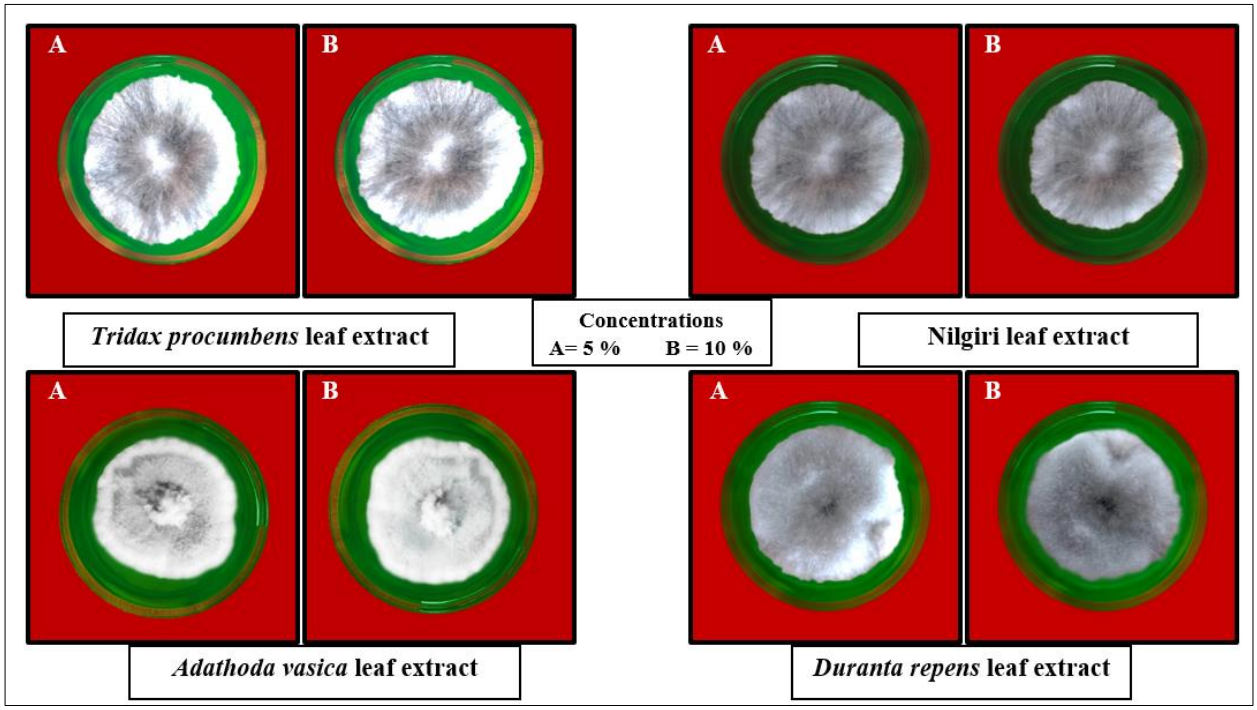


**Plate 1A:** *In vitro* evaluation of botanicals against mycelial inhibition of *C. malayensis*

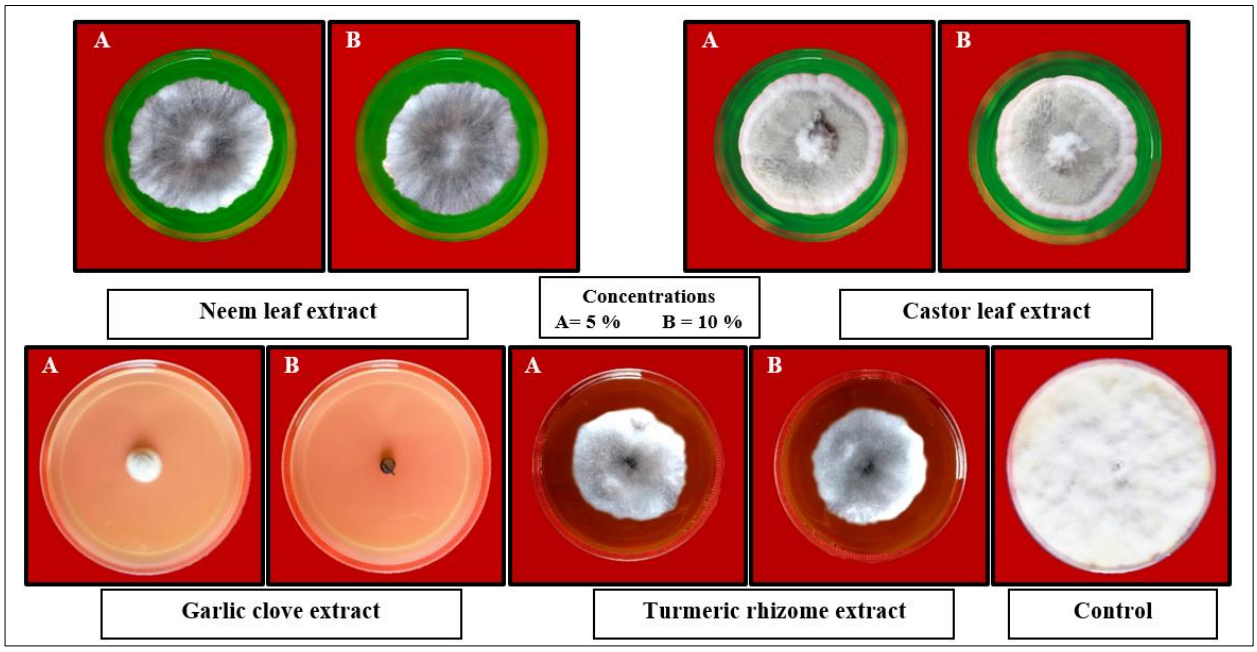


**Plate 1B:** *In vitro* evaluation of botanicals against mycelial inhibition of *C. malayensis*

## Conclusion

Out of 8 botanicals tested against *C. malayensis* complete inhibition of mycelial growth over control was recorded in garlic clove extracts at 10 per cent concentration therefore it might be a promising material to control these fungi. The next followed turmeric rhizome extracts (25.19 and 30.52%) at 5 and 10% concentrations.

The garlic plant extracts from different plant material having proven medicinal properties. The logic behind this practice is to utilize the proven efficacy of bioactive compounds possessing antimicrobial properties present in plant parts. The volatile antimicrobial substance allicin (diallyl thiosulphinate) is produced in garlic when the tissues are damaged and the substrate alliin (S-allyl-l-cysteine sulphoxide) mixes with the enzyme alliin-lyase. Allicin is readily membrane-permeable and undergoes thiol-disulphide exchange reactions with free thiol groups in proteins. It is thought that these properties are the basis of its antimicrobial action.

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