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Paras N Sangani
Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, (NAU), Navsari, Gujarat, India

Prashant B Sandipan
Main Cotton Research Station (MCRS), Navsari Agricultural University (NAU), Surat, Gujarat, India

Nirva Patel
Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, (NAU), Navsari, Gujarat, India

Twinkal D Manavadria
Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, (NAU), Navsari, Gujarat, India

Corresponding Author:
Paras N Sangani
Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, (NAU), Navsari, Gujarat, India

In vitro evaluation of different plant extracts on the growth of *Corynespora cassiicola* causing target leaf spot disease of cotton under South Gujarat of India

Paras N Sangani, Prashant B Sandipan, Nirva Patel and Twinkal D Manavadria

Abstract
Cotton (*Gossypium hirsutum* L.) stands as one of the globe's foremost fiber crops, occupying a pivotal role in both economic and social contexts. Referred to as "The white gold" or "The king of fibers," it holds the status of a premier cash crop within our country, belonging to the Malvaceae family. India, a prominent cotton producer, ranks first in terms of cultivation area and second in total cotton production globally. Cotton is cultivated across the world for its natural fiber and oil. It serves as a primary raw material for a thriving textile industry and represents one of the most ancient and vital commercial crops, second only to food grains. In the present experiment, six distinct plant extracts were assessed at concentrations of 10% and 20% against *C. cassiicola*, a cotton pathogen, in controlled *in vitro* conditions. All plant extracts exhibited substantial inhibition of the mycelial growth of the pathogen when compared to the control group. The lowest mycelial growth with the maximum% growth inhibition at 10% and 20% concentrations was recorded in Garlic bulb extract (*A. sativum*) while, the highest mycelial growth with minimum% growth inhibition at 10 percent concentration was recorded in Onion bulb extract (*A. cepa*) and at 20% concentration in Datura leaf extract (*D. stramonium*).

Keywords: *Gossypium hirsutum, Corynespora cassiicola*, target leaf spot, cotton

Introduction
Cotton (*Gossypium hirsutum* L.) holds immense significance as a primary fiber crop, playing a pivotal role in the global economic and social landscape. It has earned esteemed monikers such as "The White Gold" or "The King of Fibers". India proudly holds the title of being the largest cotton-producing nation globally, boasting an extensive cultivation area spanning 130.49 lakh hectares and an impressive annual production of 337.23 lakh bales. Within India, the state of Gujarat stands out, with cotton being cultivated over 25.49 lakh hectares and yielding a remarkable production of 87.12 lakh bales (Anonymous, 2023) [3]. In India, cotton productivity remains significantly hampered due to various constraints, with diseases being a prominent factor. Diseases are inherent components of the agro-ecosystem that necessitate continuous management based on knowledge. Cotton is susceptible to a range of diseases caused by fungi, bacteria, and viruses. The most common cotton diseases reported in India are Wilt (*Fusarium oxysporum* f. sp. *vasinfectum* (G.F. Atk.) W.C. Snyder & H.N. Hansen), Root rots (*Rhizoctonia bataticola* (Taubenh.), Verticillium wilt (*Verticillium dahliae* Kleb.), Anthracnose (*Colletotrichum gossypii* Southworth. or *C. capsici* (Syd.) Butler & Bisby), Grey mildew (*Ramularia areola* G.F. Atk.), Blackarm (*Xanthomonas campestris* pv. *malvacearum* (Pammel) Dowson), Leaf blight (*Alternaria macrospora* Zimm), Leaf curl (Cotton leaf curl virus), Corynespora leaf blight (*Corynespora cassiicola* (Berk. & M. A. Curtis) C. T. Wei, Boll rot and physiological disorders as Para wilt, Leaf reddening and sometimes leaf elongation due to improper use of weedicides etc. Corynespora leaf blight, also known as "Target spot," affects cotton (*Gossypium hirsutum* L.) and is caused by *Corynespora cassiicola* (Berk & Curt.) Wei. This pathogen isn't limited to cotton; it can also infect various other crop plants. Interestingly, the disease was first reported on soybean in Bolivia in 1994 and later on cotton in the State of Mato Grosso, Brazil, in 1995. (Mehta and Barea, 1994) [7] and Mehta *et al*., 2005 [8]. In the year 2021, according to survey results, it is evident that the disease intensity in Surat district ranged from 1.50% to 26.50%. The highest disease intensity, ranging from 0.00% to 26.50%, was observed in LRA 5166. Following closely were G. Cot. Hy. 12 BG II with a disease intensity ranging from 0.00% to 8.50%, G. Cot. Hy.

8 BG II with a range of 0.00% to 6.50%, and G. Cot. Hy. 10 BG II with a range of 0.00% to 5.50% in Choryasi taluka of Surat district (Patel *et al*., (2023)) [11]. In the context of South Gujarat, bacterial blight stands as the most prevalent and destructive disease. It has been reported to cause substantial yield losses, ranging from about 10 to 30 percent. (Kalpana *et al*., 2004 [4] and Sandipan *et al*., (2017a) [14] and Sandipan *et al*., (2017b)) [15]. Over the years, the widespread and uninterrupted use of pesticides and fertilizers has presented not only a significant risk to human health and ecosystems but has also proven to be detrimental to soil microorganisms and human well-being. Nevertheless, the production of agricultural crops remains consistently vulnerable and weakened due to attacks by pests and diseases, including insects, bacteria, fungi, nematodes, viruses, and more Plant diseases pose significant challenges to crop production, impacting both the quality and quantity of agricultural yields. These losses represent a substantial threat to global food production, with plant diseases accounting for approximately 27 to 42 percent of the losses. Without the application of effective disease management strategies, these losses could potentially double, underscoring the crucial importance of proactive measures to protect crop yields and food security (Singh, 2014, Alizadeh *et al*., 2020) [13, 2]. In recent times, a range of approaches has been employed to effectively manage and mitigate various plant pathogens, addressing the control of plant diseases. The utilization of microbial pesticides stands out as one of the most promising strategies for combatting these diseases in an environmentally friendly and safe manner. Numerous biopesticides based on bacteria and fungi have been identified and developed, showcasing great potential. Indeed, the crucial factor in harnessing the potential of microbial pesticides is their successful adoption and ongoing improvement, coupled with further advancements in enhancing these bioagents. Furthermore, there is a growing trend among consumers who are increasingly concerned about pesticide-free, safer food options. This trend has led to the emergence of eco-friendly strategies for managing plant diseases, aligning with the broader societal shift towards more sustainable and environmentally conscious agricultural practices. In today's agricultural practices, the use of various plant extracts has become commonplace on a commercial scale. Consequently, this experiment was designed to explore the effectiveness of plant extracts in controlling *C. cassiicola* disease in cotton under controlled *in vitro* conditions.

Materials and Methods

Fresh plant parts were collected and meticulously washed, initially with tap water and subsequently with distilled water. One hundred grams of the freshly gathered sample were then finely chopped and crushed using a surface-sterilized pestle and mortar, with the addition of 100 ml sterile water at a ratio of 1:1 (w/v). The resulting extract was meticulously filtered through two layers of muslin cloth and subsequently subjected to centrifugation at 10,000 rpm. The final filtrate obtained in this manner served as the stock solution for the experiment (Photo 1) (Sindhan *et al*., 1999) [12].

To study the antifungal mechanism of plant extracts, Poisoned food technique was used (Nene and Thapliyal, 1973) [10]. In this method, 10 ml and 20 ml of the stock solution were combined with 90 ml and 80 ml of sterilized molten Potato Dextrose Agar (PDA) medium, respectively, to achieve concentrations of 10 percent and 20 percent. The mixture was thoroughly agitated to ensure even distribution of the extract within the medium. To prevent bacterial contamination, a small amount of streptomycin was introduced into each flask before plating (Hiremath *et al*., 2020) [4].

Each Petri plate was inoculated with a 5 mm disc taken from a seven-day-old culture of *C. cassiicola*, using a sterilized cork borer for precision. These inoculated Petri plates were then placed in an incubator at a temperature of 27±2°C and left to incubate for seven days. To serve as a control, a separate set of Petri plates were maintained where the growth medium was not supplemented with any of the plant extracts. The experiment followed a completely randomized design with three repetitions. After seven days of incubation, the colony diameter was measured for analysis.

The effectiveness of botanicals was quantified as the percentage inhibition of mycelial growth compared to the control. This was determined using the following formula given by Asalmol *et al*. (1990) [1].

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Inhibition of mycelial growth (%)
C = Radial growth of mycelial in control (mm)
T = Radial growth of mycelial in treatment (mm)

Table 1: List of plant extracts used

Tr. No.	Treatments	Plant part used	Concentration (%)	
T_1	Neem extract (*Azadirachta indica* A. Juss.)	Leaves	10	20
T_2	Datura extract (*Datura stramonium* L.)	Leaves	10	20
T_3	Ginger (*Zingiber officinale* Roscoe)	Rhizome	10	20
T_4	Onion extract (*Allium cepa* L.)	Bulb	10	20
T_5	Turmeric (*Curcuma longa* L.)	Rhizome	10	20
T_6	Garlic extract (*Allium sativum* L.)	Bulb	10	20
T_7	Absolute control (without treatment)	-	-	-

Photo 1: Different types of plant extracts used in the experiment

Design: Completely Randomized Design
Treatments: 13
Repetitions: 3
Method: Poisoned food technique
Location: Department of Plant Pathology, Post Graduate Laboratory, N. A. U., Navsari, Gujarat.

Results and Discussion

Plant extract of six different plant parts were evaluated *in vitro* at two concentrations 10 and 20% against *C. cassiicola* and the results obtained on its colony diameter and mycelial growth inhibition are presented in (Table 2). Result revealed that all the six plant extracts tested were fungistatic/antifungal to *C. cassiicola*, which significantly reduced the colony diameter and increase its inhibition over untreated control. The colony diameter was decreased and its inhibition was increased in relation to the concentrations of the plant extract tested (Table 2).

At 10% concentration the colony diameter of the *C. cassiicola* ranged from 50.80 mm in Garlic bulb extract (*Allium sativum* L.) to 82.07 mm in Onion bulb extract (*Allium cepa* L.) as compared to 90.00 mm in untreated control. The minimum colony diameter was recorded in the Garlic bulb extract with 50.80 mm which, was significantly superior over all the treatments and was found at par with Ginger rhizome extract (*Zingiber officinale* Roscoe) with 51.50 mm. Next in order of merit, the Neem leaf extract (*Azadirachta indica* A. Juss.) with 55.06 mm colony diameter was recorded and however, the maximum colony diameter was recorded in onion bulb extract with 82.07 mm followed by Datura leaf extract (*Datura stramonium* L.) with 80.47 mm and Turmeric rhizome extract (*Curcuma longa* L.) with 71.90 mm (Table 2, Photo 2).

Maximum mycelial growth inhibition at 10% concentration was recorded in Garlic bulb extract with 43.56% followed by Ginger rhizome extract with 42.78% and Neem leaf extract with 38.81%. The least inhibition was observed in Onion bulb extract with 8.81% followed by Datura leaf extract with 10.59% and Turmeric rhizome extract in the tune of 20.11% (Table 2, Fig. 1).

At 20% concentration, the colony diameter was ranged from 10.77 mm in Garlic bulb extract to 69.73 mm in Datura leaf extract. The least colony diameter was recorded in Garlic bulb extract with 10.77 mm which, was followed by the plant extracts *viz*., Neem leaf extract 19.13 mm, Turmeric rhizome extract 22.50 mm, Ginger rhizome extract 29.20 mm and Onion bulb extract with 39.20 mm, respectively. However, the maximum colony diameter was recorded in Datura leaf extract with 69.73 mm (Table 2, Photo 3).

In case of mycelial growth inhibition, Garlic bulb extract showed the maximum mycelial growth inhibition of 88.04% in comparison to all the other plant extracts. The next effective plant extracts at 20% concentration in order of inhibition were Neem leaves extract with 78.74%, Turmeric rhizome extract with 75.00%, Ginger rhizome extract with 67.56%, Onion bulb extract with 56.44% and Datura leaf extract with 22.52% was found least effective in inhibiting the mycelial growth of *C. cassiicola* (Table 2, Fig. 1).

The above research findings are in agreement with the research results of Lakshmanan (1990) [6] tested ten plant extracts *in vitro* against the *C. cassiicola* of cotton and found that garlic cloves were the most effective in inhibiting mycelial growth by 95.80%.

Manju *et al*. (2014) [9] determined that garlic bulb extract exhibited the highest efficacy in inhibiting mycelial growth, achieving an impressive rate of 60.50% inhibition at a concentration of 10 percent. This result surpassed the performance of all other tested plant extracts. Additionally, neem seed kernel and onion bulb extracts demonstrated significant antifungal properties, effectively inhibiting mycelial growth by over 50 percent when compared to the control group, in the case of *C. cassiicola* affecting rubber crops. Conversely, the datura leaf extract exhibited the lowest inhibition rate at 31.20 percent. These findings highlight the superiority of garlic bulb extract and the positive impact of neem seed kernel and onion bulb extracts in controlling *C. cassiicola*, particularly in the context of rubber cultivation.

Hiremath *et al*. (2020) [4] evaluated efficacy of six different plant extracts against *C. cassiicola*, the causal agent of target leaf spot in soybean, was investigated. The results revealed that Garlic clove extract exhibited the highest mycelium inhibition, displaying an impressive rate of 73.72%. This inhibition was statistically superior to all other treatments. Conversely, the Datura leaf extract displayed the least inhibition of mycelial growth, with a rate of only 12.74%. These findings underscore the substantial antifungal potential of Garlic clove extract and highlight the limited effectiveness of Datura leaf extract in controlling *C. cassiicola* in soybean.

Table 2: *In vitro* efficacy of different plant extracts against the colony diameter and mycelial growth inhibition of *Corynespora cassiicola*

Tr. No.	Treatment	Plant part used	Colony Diameter (mm)		Mycelial growth inhibition (%)	
			10%	20%	10%	20%
T_1	Neem leaf extract	Leaves	55.06	19.13	38.53* (38.81)	62.59 (78.74)
T_2	Datura leaf extract	Leaves	80.47	69.73	18.95 (10.59)	28.31 (22.52)
T_3	Ginger rhizome extract	Rhizome	51.50	29.20	40.85 (42.78)	55.28 (67.56)
T_4	Onion bulb extract	Bulb	82.07	39.20	17.22 (8.81)	48.70 (56.44)
T_5	Turmeric rhizome extract	Rhizome	71.90	22.50	26.64 (20.11)	60.02 (75.00)
T_6	Garlic bulb extract	Bulb	50.80	10.77	41.30 (43.56)	69.80 (88.04)
T_7	Absolute control	-	90.00	90.00	0.62 (0.00)	0.62 (0.00)
	SEm±	0.64	0.64	1.06	0.642	0.82
	CD at 5%	1.96	1.96	3.26	1.96	2.53
	CV%	4.24	4.24	4.60	4.24	3.08

*****Figures outside the parentheses are arcsine transformation values where in parentheses are original values and average of three repetitions

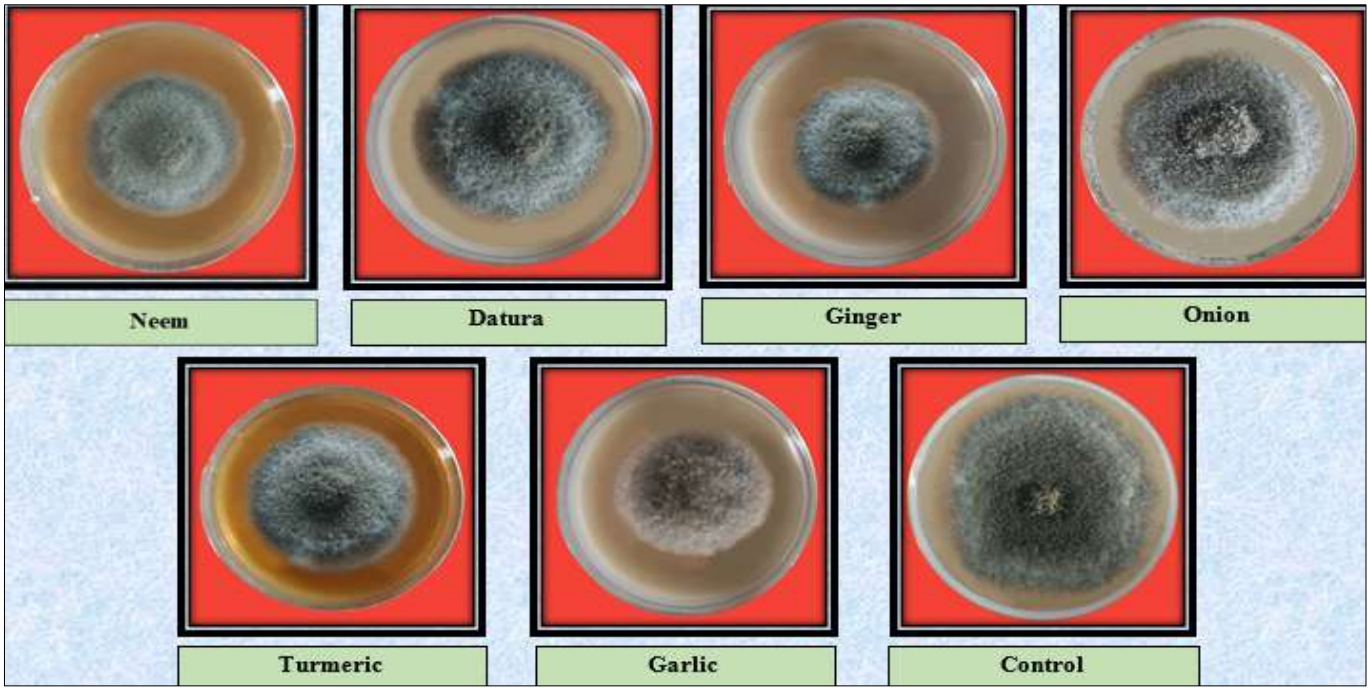

Photo 2: *In vitro* efficacy of 10% plant extract on the growth of *Corynespora cassiicola*

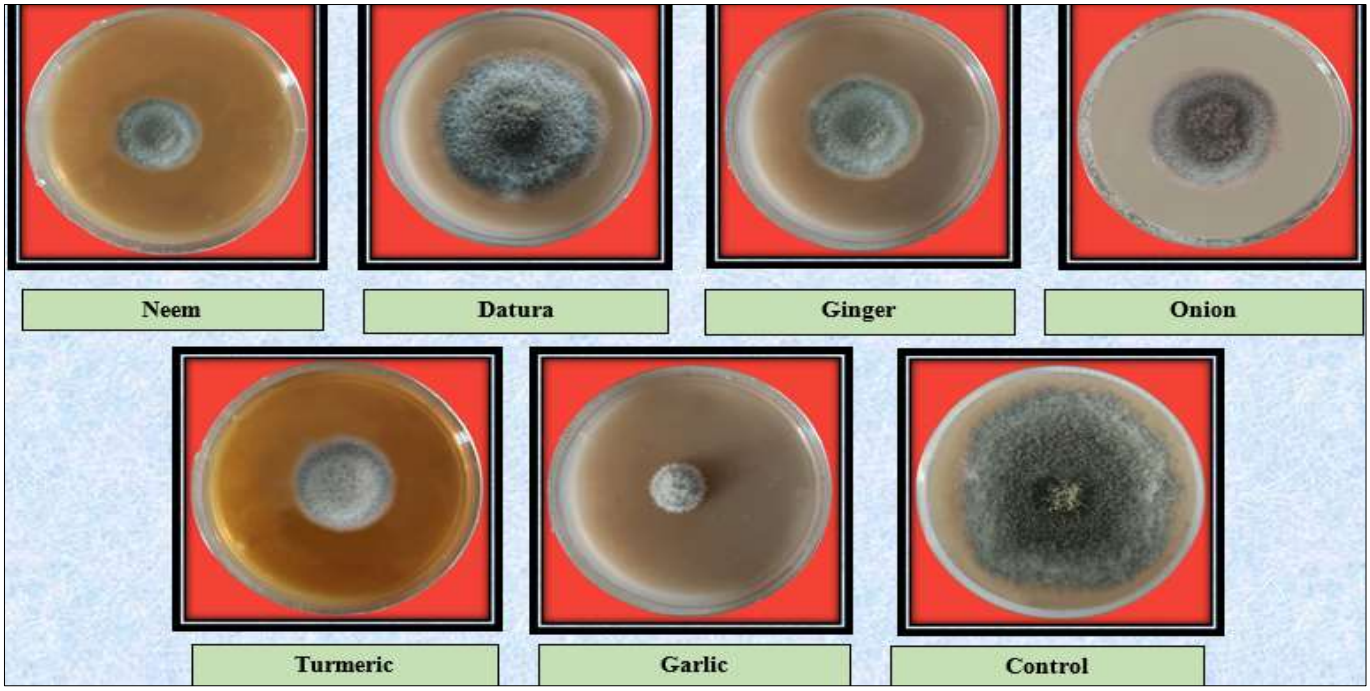

Photo 3: *In vitro* efficacy of 20% plant extract on the growth of *Corynespora cassiicola*

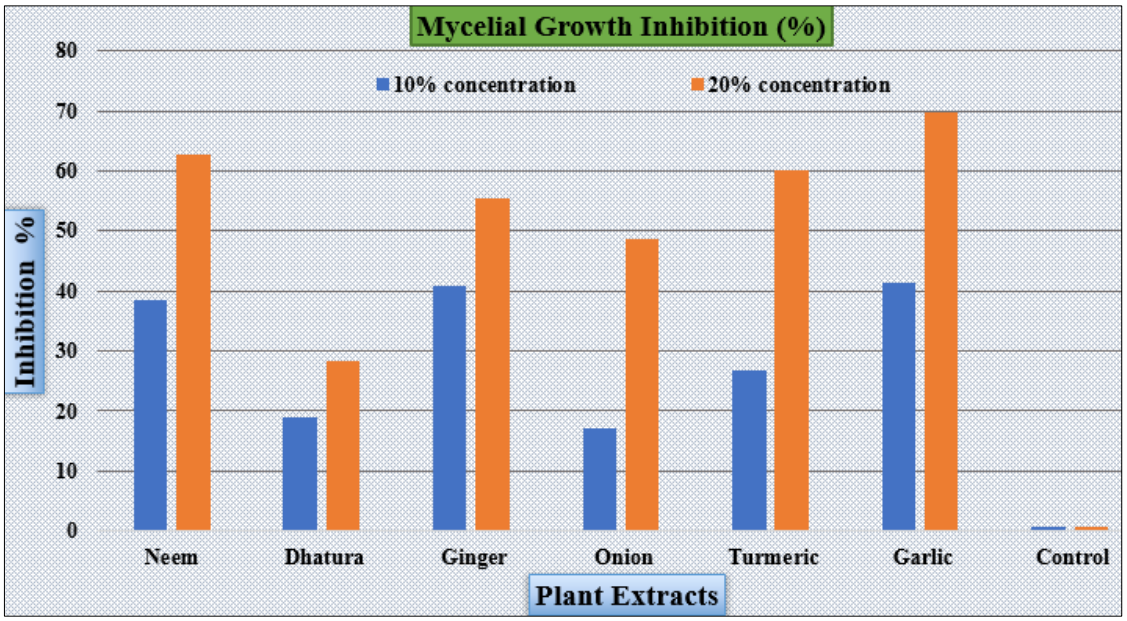

Fig 1: Effect of plant extracts on the mycelial growth inhibition of *Corynespora cassiicola*

Conclusion

The experiments conducted have led to the conclusion that target leaf spot is a severe and highly destructive disease affecting cotton, characterized by symptoms such as small, round to irregular, dark red lesions that appear in abundance on cotton plant leaves. The findings of the experiments make it evident that garlic bulb extract, particularly at concentrations of 10 percent and 20 percent, proved to be the most effective plant extract in controlling *C. cassiicola*, the causal agent of this disease.

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