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Unveiling a symptomatic disease in custard apples: Effective management strategies against fruit degradation

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Abstract

Annona squamosa is a tropical fruit susceptible to various diseases that significantly impact its yield and quality of the fruits. *Colletotrichum* spp. rank among the top ten most destructive fungal pathogens globally, occupying the 8th position due to their significant impact on agriculture. These fungi are hemibiotrophic in nature, with over 1,000 known species, and most crops are susceptible to at least one of these. They cause various diseases, including leaf spots, blights, and fruit rots. Symptoms manifest on horticultural crops' leaves, flowers, fruits, and branches, often resulting in substantial yield losses. *Colletotrichum* species exhibit a latency mechanism that can exacerbate damage during post-harvest storage, leading to up to 80 % production losses in some instances. Experiments were carried out in Randomized Block Design with seven treatments and three replications at the C1 block of Regional Research Station, TNAU, Aruppukottai, Virudhunagar District, Tamil Nadu, to identify and manage the pathogens causing leaf spot and fruit rot in custard apples. Pathogens isolated from the rotten fruit rind and mesocarp were identified as *Colletotrichum siamense* (Acc No: OM736073) and *Colletotrichum gloeosporioides* (Acc No: OM736066), respectively, through morphological and molecular analyses. The isolated DNA of the respective pathogens were amplified using PCR with ITS 1 & 4 primers. Then, the amplified product (550bp) was sequenced and submitted to the NCBI website. Pathogenicity tests confirmed these isolates as the causative agents for the leaf spot and fruit rot symptoms. Field experiments were conducted for two consecutive years to evaluate the efficacy of various treatments in managing leaf spot and fruit rot diseases. Before spraying, the leaf spot and fruit rot incidence are 77 and 27, respectively, in the control plot. The combined fungicide formulation, Tebuconazole 25 % + Trifloxystrobin 50 % WG (0.1 %), showed the highest disease reduction (60.44 %) and yield enhancement (15.50 kg/tree). The spraying of neem seed kernel extract (5 %) and *Bacillus subtilis* (0.5 %) also resulted in significant disease reduction and yield improvement. The study highlights the importance of combining chemical and biocontrol agents with integrated disease management strategies to manage custard apple diseases effectively. Neem products are always safe for the environment, Humans and animals and when they are used in correct concentration they won't leave any residues. Likewise, biocontrol agents are target-specific and never harm the beneficial microbes in soil and the plant system.

Keywords

Anthracnose; *Colletotrichum gloeosporioides*; *Colletotrichum siamense*; custard apple; molecular identification; systemic fungicides

Introduction

Annona squamosa, known as custard apple or sugar apple, is an evergreen shrub belonging to the family Annonaceae, well-suited for cultivation in tropical and semi-arid regions. It grows best in sandy, clay, and black soil with good drainage. Tropical America and the West Indies are the probable origin of this fruit crop (1). India is one of the largest custard apple producers, cultivated in Maharashtra, Andhra Pradesh, Tamil Nadu, and Karnataka. Maharashtra ranks first in production, contributing 31 percent of the total output. In Tamil Nadu, key districts involved in custard apple cultivation include Dindigul, Tiruvannamalai, Kanyakumari, Coimbatore, Namakkal, Krishnagiri, Salem, Dharmapuri and Tirunelveli (2). The genus of the *Annona* contains approximately 170 species, among which *A. reticulata* (common custard apple), *A. muricata* (soursop), *A. squamosa* (sweetsop), *A. cherimola (cherimoya)* and *A. glabra* (alligator apple) are significant (3).

Custard apple is rich in soluble fibres and antioxidants, which act as natural laxatives, help to remove toxins and improve bowel movement (4). This fruit is rich in polyphenolic compounds, which also help combat chronic diseases like diabetes, cardiovascular disease, and cancer. Major diseases affecting custard apple production include leaf spot, anthracnose, powdery mildew, phytophthora fruit rot, root rot and bacterial canker. Among these, leaf spot and fruit rot (Anthracnose) caused by *Colletotrichum* spp. This significantly reduces the market value of the fruits, and it is a common practice for farmers to remove rotten fruits before selling them. The yield loss may vary from 20–80 %, depending on the infection severity, environmental factors and *Annona* cultivars (5). Integrating fungicides with biocontrol agents reduces the risk of developing resistance to pathogens. As the fungicide spray is planned well before the fruits' maturation, the fungicide residues will not be deposited in any parts of the plant system.

Biocontrol agents, namely *Bacillus subtilis*, didn't exhibit any toxicity in the plant system or on the beneficial microflora that survived both inside and outside the plant system. Likewise, other botanical formulations, namely neem seed kernel extract and thyme oil, are eco-friendly and do not show any residual toxicity in the plant system. Here, an attempt was made to identify and characterize the pathogens responsible for various symptoms in custard apples (6). Field trials were conducted to manage the disease using systemic fungicides, neem seed kernel extract, botanicals and bio-control agents.

Materials and Methods

The research was conducted at the Regional Research Station, Aruppukottai, Virudhunagar District, Tamil Nadu, under the Indian Council of Agricultural Research-All India Coordinated Research Project on Arid Zone Fruits during 2022–24. Experimental trials were conducted in the orchard, which had the custard apple variety APK 1 (CA) established in 2007–08. The region experiences a tropical climate from October to February, typically recording moderate temperatures between 18 °C and 28 °C. High relative humidity, ranging from 75 to 87 %, was recorded during this period compared to the other seasons. The recorded rainfall during the season ranged between 600 mm and 800 mm. These environmental factors during the experimental period were considered, as they could have influenced disease incidence and affected the custard apple's yield.

Symptoms of *Colletotrichum* spp. on custard apple

Symptoms, such as leaf spot, blight, dieback, small necrotic spots and patches of black lesions, marginal drying of leaves with shot hole symptoms, necrotic lesions on unopened flower buds and fertilized flowers, as well as mummified and rotten fruits, were noticed in the field level. In unopened buds, this results in drying and dehiscence of petals, while fertilized buds become corky, hard and mummified without flesh. Additionally, the pathogen affects immature fruits, producing black lesions on the fruit skin, leading to fruit rot (Fig. 1). The severity of these symptoms was rated using a 0 to 9 scale, where 0 indicates no symptoms, 1-2 represents slight symptoms, 3-4 moderate symptoms, 5-6 moderate to severe symptoms, 7-8 severe symptoms, and 9 indicates very severe symptoms, including extensive fruit rot and complete necrosis of affected tissues (7).

Fig. 1. Symptoms of *Colletotrichum* sp. affected parts of custard apple.

Isolation and morphological confirmation of pathogen

Infected symptomatic samples (spot and blight-affected leaves, dried twigs, unopened flower buds, corky fruits, and rotten fruits) were collected. Each sample was cut into small pieces of 1 cm and surface sterilized with sodium

hypochlorite (NaOCl 1 %), then washed with sterile water. Then, the pieces were inoculated on potato dextrose agar medium. The plates were incubated at room temperature for 3–7 days until the fungal mycelium growth was observed. Mycelium was purified based on the colour of the PDA to obtain a pure culture (7). The cultures isolated from the rind and mesocarp were coded as Skin CA (APK)-1 and Fruit CA (APK)-1. These two isolates were used for further confirmation studies. All the symptomatic parts, after incubation, produced white to grey-coloured mycelium. Each pure culture was observed under an image analyzer with 40X magnification, and photographs were taken to study the conidial morphology. The isolation procedure was repeated three times to confirm the morphological identity of the pathogens.

Proving the pathogenicity

A pathogenicity test was conducted using detached leaves of custard apple. Healthy leaves from the APK 1 (CA) variety were collected and surface sterilized by dipping them in 70 % ethanol for 30 sec. Then, they were rinsed three times with sterile water and dried on sterilized filter paper. Mycelial plugs (9 mm) were taken from 7-10 days cultures of *Colletotrichum* sp. isolated from leaf spot symptoms. Before inoculation, five holes were made on the symmetric side of the leaves using the pinpricking method (8). Control leaves were inoculated with non-colonized agar plugs. A pathogenicity test on fruits was conducted via standard procedure (8). Detached immature custard apple fruits were used for this study. Fruits were sterilized with sodium hypochlorite (1 %) and ethanol (70 %), then pinpricked and inoculated with mycelial bits on the wounded areas. The pathogenic isolate cultured from fruit pulp was used for this inoculation.

Molecular confirmation of pathogen

Pathogens isolated from the rind (skin) and custard apple pulp were cultured in PDA broth. After seven days of incubation, mycelial mats of two pathogens were collected and dried on sterilized blotter paper. DNA extraction was performed using the CTAB extraction method (9). For PCR amplification, a reaction mixture of 10 µl was prepared, comprising 5 µl of master mix (including 0.25 mM dNTP, 1.5 mM $MgCl_2$, Taq polymerase and buffer), 1 µl each of forward and reverse primers (ITS 1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS 4: 5'-TCCTCCGCTTATTGATATGC-3') µl of DNA, and 1 µl of sterile distilled water. Instead of DNA, one microlitre of sterile water was used as a negative control. PCR was conducted using a master cycler with the following program: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation (94°C for 1 minute), annealing (46 °C for 1 minute), extension (72 °C for 1 minute), and a final extension at 72 °C for 10 minutes. Gel electrophoresis was performed on a 1.5 % agarose gel containing 3 microlitres of EtBr dye, and the PCR products (550 bp) were visualized and documented under UV light. PCR-amplified products of the two pathogens were sent to Eurofins Genomics India Pvt Ltd in Bangalore, India, for sequencing, and the sequences were submitted to NCBI, which received the accession numbers (10) (Fig. 2).

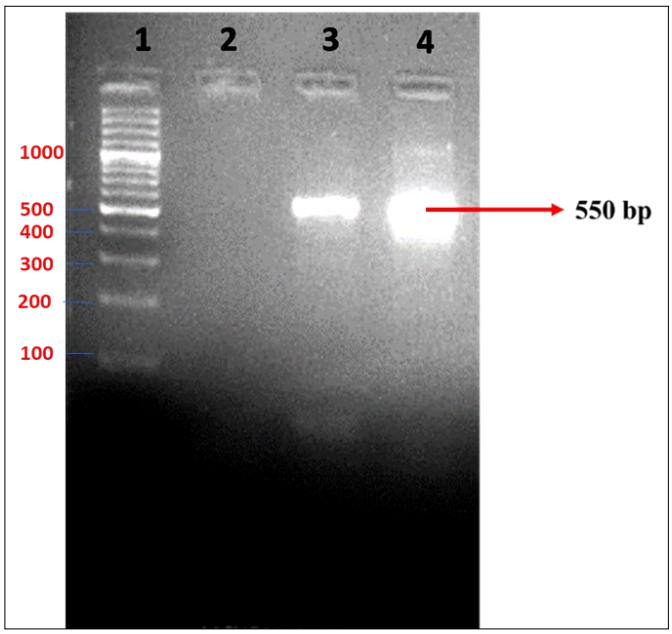

Fig. 2. PCR amplification of pathogenic isolates of custard apple fruit. The samples were loaded into the gel electrophoresis lanes as follows: Lane 1 - 100 bp ladder; Lane 2 - water as a negative control; Lane 3- a DNA sample from the pathogen (rind CA (APK)-1); Lane 4 -a DNA sample from the pathogen (fruit (APK)-1).

Phylogenetic analysis

The obtained sequences were assembled into complete contigs using DNA Baser V.4 software. These contigs were aligned using the ClustalW method in Bio-Edit software and the resulting sequences were submitted to the NCBI GenBank. Homologous sequences were identified through BLAST analysis on the NCBI website. Subsequently, the sequences of our pathogenic isolates and 13 reference strains from GenBank were used to construct a phylogenetic tree. An evolutionary history was inferred using the Neighbor-Joining method (11). The Neighbor-Joining (NJ) method was selected for its computational efficiency and suitability for inferring phylogenetic relationships based on distance data. This method is particularly advantageous when handling large datasets, as it minimizes computational time while maintaining accuracy. Additionally, the NJ method is widely used in phylogenetic analysis due to its ability to produce reliable tree topologies without assuming a constant rate of evolution, making it appropriate for our dataset. The bootstrap consensus tree inferred from 1000 replicates represents the evolutionary history of the taxa analyzed.

Branches corresponding to partitions reproduced in less than 50 % of bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (11). This analysis involved 16 nucleotide sequences. Codon positions included were 1st, 2nd, 3rd and non-coding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 557 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (11–12).

Management of leaf spot and fruit rot of custard apple under field condition

The experiment was conducted at the C1 block of RRS, Aruppukottai, in the custard apple (Var. APK 1 (CA)) orchard during the Rabi season for two consecutive years (2022–24). As per the recommended practices for custard apple cultivation, an annual dose of 100:75:75 g of NPK per tree and 20 kg of FYM (Farmyard Manure) were applied to ensure proper plant nutrition and health. The experiment was designed in a Randomized Block Design (RBD) with 8 treatments and 3 replications. Two trees were taken for a single replication. The spacing between plants was maintained at 5 m × 5 m and a similar distance was followed between replications to ensure uniform growth conditions and proper experimental layout. Leaf spot incidence was observed during the peak monsoon season. Treatments were applied in December. The first application was done immediately after the disease appearance, followed by a second application fifteen days later. The 'Percent Disease Index (PDI) of the leaf spot and fruit rot was calculated using a 0-9 grade scale (7). Data from both years were pooled for analysis.

Statistical analysis

Mean differences between treatments were statistically evaluated using the methods described [13]. Analysis of variance (ANOVA) was performed using SPSS software (version 25), followed by Duncan's Multiple Range Test at a 5 % significance level.

Results and Discussion

Morphological confirmation of pathogens isolated from various symptomatic parts of the tree

In our research, *Colletotrichum* spp. It causes many symptoms in custard apples, including leaf spots, blight, dieback, unopened flower buds, and corky and rotten fruits. Based on morphology and molecular observations, *Colletotrichum gloeosporioides* (Fruit (APK)-1) and *C. siamense* (CA (APK)-1) are the species causing leaf spot and fruit rot of custard apple. Grey-coloured septate mycelia with hyaline cylindrical conidia were observed in cultures isolated from each symptomatic part under 40X magnification using an Image analyzer (Fig. 3). Morphologically, it was identified as *C. gloeosporioides.* The mycelium is greyish white, covering the petriplate within 7 days, later turning grey in colour and septate. Occasionally, a conidium can be observed at the tip of the conidiophore. The pathogen also produces chlamydospores. The mycelium spreads rapidly, immersed and branched. The pathogen produces an orange-coloured conidial mass.

Morphologically, it is challenging to differentiate *C. siamense* from *C. gloeosporioides* because these two species produce white and grey-coloured mycelium and cylindrical conidia. While observing under the microscope, cylindrical conidia are observed from each symptomatic part. Based on morphology, it was clear that the pathogens isolated from the above symptomatic parts belong to *Colletotrichum* spp (14).

Our results confirm that anthracnose is a highly devastating disease in custard apples (14). Due to this incidence, blackened fruits were formed. During 2005–06, one-third of custard apple orchards in Pune were severely affected by this disease. The loss ranged between 60–70 %. Diseased plant parts like twigs, bark, stem piths, branches, leaves, fallen flower buds, and fruits show typical symptoms collected from heavily infected plants. From the above symptomatic parts, grey-coloured mycelium was isolated and cylindrical conidium was noticed in each culture.

Proving pathogenicity

The inoculated custard apple leaves produced dark lesions after the incubation period. Likewise, dark lesions with

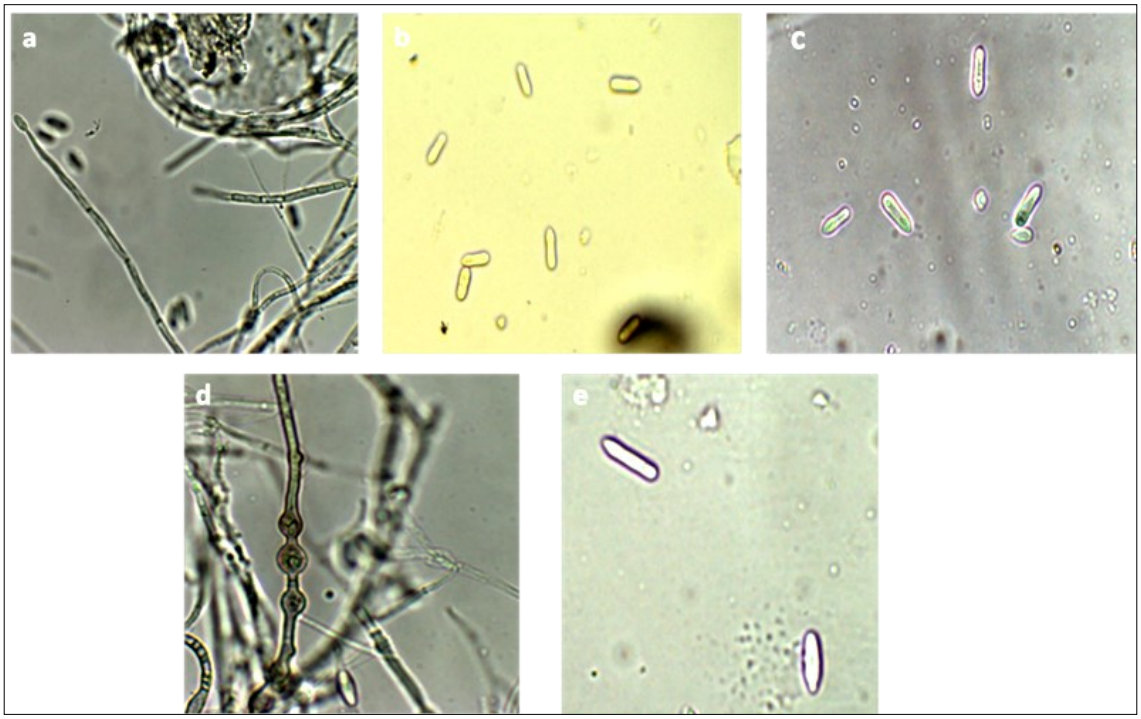

Fig. 3. Microscopic observation under 40 X.

dark brown necrotic spots were observed on the inoculated fruits. The mycelium isolated from the leaves and fruits was grey-coloured, and cylindrical conidia with round tips were observed in the above pathogenic cultures, which resembled the original pure cultures isolated from the initial symptomatic parts of leaves and fruits. This confirmed Koch's postulates. Our results align with earlier findings that successfully demonstrated the pathogenicity of *C. gloeosporioides* (8). They attempted to study the host range of *C. gloeosporioides*, previously isolated from the custard apple tree and already affected by typical symptoms like leaf spots die back and fruit rot. Around 115 plants belonging to a wide range of different families were chosen. Sixty-day-old plants of all the above species were inoculated separately by the Mycelial Bit Inoculation method (MBIM) and Micro Droplet Inoculation Technique (MBIT). In the case of the second method, spore suspension (10^5 conidia /ml) was used. Control plants were maintained in each plant species without inoculation. Generally, symptom expression was noticed on the 10^{th} day after inoculation. This pathogen infected 33 different plant species out of the 115 inoculated, including chilli, avocado, cashew nut, guava, papaya, mango, pomegranate, tomato, bell pepper, nutmeg, and curry leaf, among others. This indicates that the pathogen has a wide host range (8)

Colletotrichum gloeosporioides is a facultative parasite belonging to Melanconiales. The conidia of the pathogen ranged in shape from ovoid to oblong, with some exhibiting a dumbbell shape and a slight curve (15). Typical symptoms caused by this pathogen on plants include anthracnose, sunken necrotic lesions on petioles and leaves, and mummified inflorescences and flower bracts. The pathogen results in significant losses for fruit growers in quality and quantity. Understanding the interaction between the existing and emerging populations of host species and varieties is crucial.

Fourteen *Colletotrichum* isolates (Cg 1-14) were separated from diseased specimens of various fruits, mainly pomegranate, custard apple, papaya, guava, mango, and lime. Isolates were also obtained from crops like chilli, onion, garlic, and jasmine. Among these, Cg-5 from mango and Cg-2 from custard apple were identified as particularly aggressive. These two isolates affected various hosts, including fruits, vegetables, jasmine, turmeric, onion, and garlic. Interestingly, isolates from the family *Amaryllidaceae* (onion and garlic) showed a notable host specificity, only infecting crops within the same family. In contrast, other *Colletotrichum* isolates from different host species exhibited a broader host range.

This host specificity in isolates from the *Amaryllidaceae* family could be attributed to several factors, including genetic and biochemical differences that might influence the pathogen's ability to recognize and infect specific hosts. Genetic factors, such as particular effector genes facilitating host-pathogen interactions, may be critical in determining the narrow host range. Additionally, the pathogen may produce certain enzymes or secondary metabolites that specifically interact with the biochemical makeup of the host plants within the *Amaryllidaceae* family. Future studies focusing on the genetic makeup of these isolates, such as identifying effector genes and understanding their interactions with host plant receptors, would provide more insight into the mechanisms behind this host specificity.

Molecular confirmation of Colletotrichum spp.

Pathogens isolated from the rind (CA (APK)-1) (3) and fruit (Fruit (APK)-1) (4) produced an amplified product of 550 bp. While BLAST searching, the pathogens were identified as *Colletotrichum siamense* (rind) OM736073 and *Colletotrichum gloeosporioides* (fruit) OM736066. *Colletotrichum siamense* was recovered from the fruit rind, and *C. gloeosporioides* was the pathogen that caused fruit rot in the mesocarp.

Pathogens isolated from the rind (CA (APK)-1) (3) and fruit (Fruit (APK)-1) (4) produced amplified products of 550 bp. DNA sequencing was performed to confirm the morphological identification of the pathogens. The sequences obtained were subjected to BLAST searching, which identified the pathogen from the rind as *Colletotrichum siamense* (rind) OM736073 and the pathogen from the fruit as *Colletotrichum gloeosporioides* (fruit) OM736066. *Colletotrichum siamense* was recovered from the fruit rind, whereas *C. gloeosporioides* was identified as the pathogen causing fruit rot in the mesocarp.

Phylogenetic analysis

BLASTn analysis of the sequence data of our isolate CA (APK)-1 revealed 100 % similarity with reference isolates *Colletotrichum siamense* GM710 (OQ842306.1) and *C. siamense* GM79 (OQ842305.1) from China.

Likewise, the culture Fruit (APK)-1 showed 100 % similarity with the reference isolate *C. gloeosporioides* OR378793 from Kerala, India. The phylogenetic analysis of ITS sequences showed that isolates OM736073.1 (*C. siamense* isolate Skin CA(APK)-1) and OM736066.1 (*C. gloeosporioides* isolate Fruit (APK)-1) formed a distinct cluster, showing 100 % similarity with other *C. gloeosporioides* clusters, such as KX022506.1 from Malaysia and OR908922.1 from India. The outgroup used in this study was *Lasiodiplodia parva* CBS 456.78 (NR_111265.1) (Fig. 4).

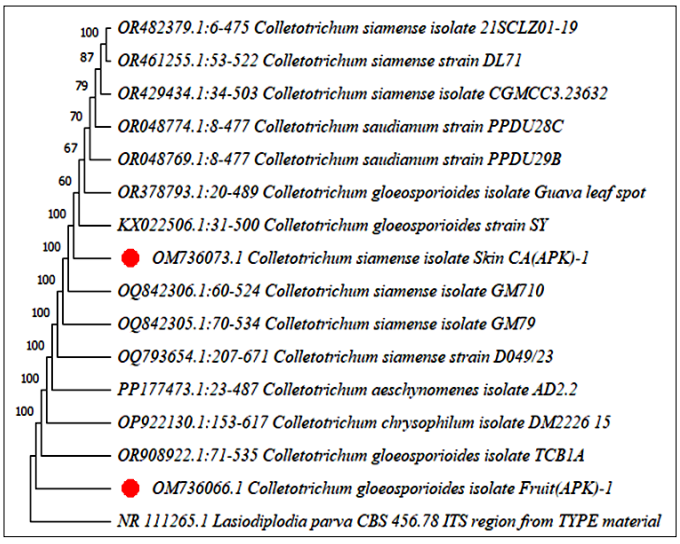

Fig. 4. Neighbour-joining tree showing the relationship of *Colletotrichum* spp.

In the field experiment, the combined formulation of fungicides Tebuconazole 25 % + Trifloxystrobin 50 % WG (0.1 %) showed the best results in reducing leaf spot disease incidence by 63.76 % and achieving the highest yield of 15.50 kg/tree followed by neem seed kernel extract (5 %), which showed a 51.20 % reduction in disease incidence (yield–13.00 kg/tree)and *Bacillus subtilis* (Bbv57) (0.5 %), which resulted in a 41 % reduction in disease incidence (yield: 12.50 kg/tree) (Table 1). The data were analyzed statistically using one-way Analysis of Variance (ANOVA) to compare the effectiveness of different treatments, followed by Tukey's HSD post-hoc test, which confirmed that the combined formulation of fungicides significantly outperformed the other treatments ($p < 0.05$).

The efficacy of fungicides, botanicals, neem-based products, and the bacterial biocontrol agent *Bacillus subtilis* was consistent across two years of field experiments (2022–2024). Among the treatments tested, Tebuconazole 25 % + Trifloxystrobin 50 % WG performed the best, significantly reducing disease incidence to a PDI of 4.5, the lowest among all treatments, and achieving the highest yield of 10.92 t/ha compared to the control plot (3.02 t/ha). Statistical analysis using ANOVA confirmed the significant differences between treatments ($p < 0.05$), and Tukey's HSD post-hoc test identified Tebuconazole + Trifloxystrobin as significantly more effective than the other treatments.

This combination of fungicides also proved effective at lower concentrations, suggesting its suitability as a preventive strategy against anthracnose under field conditions. The results align with previous studies on anthracnose management in pomegranate and similar fruit crops, where Tebuconazole-based formulations consistently outperformed other treatments in reducing fruit rot and improving yield. However, variations in pathogenic behaviour and fungicide sensitivity across crops, environmental conditions, and host-pathogen interactions must be considered to validate these findings further.

Table 1. Effect of botanicals, bio control agent and fungicides on the incidence of leaf spot and fruit rot of custard apple (Pooled mean 2022–24).

Treatments details	Leaf spot incidence*	Per cent reduction over control	Fruit rot incidence*	Per cent reduction over control	Yield* (kg/tree)
T_1-Tebuconazole 25 %+ Trifloxystrobin 50 % WG (0.1 %)	30.48^{e}	60.44	4.72^{e}	82.52	15.50^{a}
T_2-Tebuconazole 25.9 % w/w EC (0.1 %)	56.39^{b}	26.81	11.90^{c}	55.92	10.00^{cd}
T_3-Metiram 55 % + Pyraclostobin 5 % WG (0.1 %)	56.15^{b}	27.11	10.51^{c}	61.07	11.00^{bc}
T_4-*Bacillus subtilis* (Bbv57) (0.5 %)	45.45^{c}	41.10	7.83^{d}	71.00	12.50^{b}
T_5-Thyme oil 5EC (1 %)	46.65^{c}	39.50	6.55^{d}	75.74	11.75^{bc}
T_6-NSKE 5 %	37.64^{d}	51.20	5.92^{d}	78.07	13.00^{b}
T_7-Thiophanate methyl 70 % WP (0.1 %)	55.67^{b}	27.80	20.67^{b}	23.44	11.50^{bc}
T_8-Control	77.07^{a}	-	27.00^{a}	-	8.50^{d}
Sed	3.50		2.55		0.99
CD(*P*=0.05)	7.52		5.48		2.11
CV %	8.47		26.36		10.33

*Means followed by the same letter differ non-significantly at P ≤ 0.05 according to DMRT, *Values are mean of three replications.

Although the combined fungicide formulation was adequate, its excessive use may pose environmental risks, such as soil and water contamination, non-target effects on beneficial organisms and potential toxicity to humans and wildlife. These risks highlight the need for safer, alternative treatments. The current study evaluated neem seed kernel extract (5 %) and *Bacillus subtilis* (Bbv57) as environmentally sustainable alternatives. Both treatments demonstrated promising results, while neem seed kernel extract reduced disease incidence by over 50 %, making it a viable option for integrated disease management with minimal environmental impact.

Monitoring the pathogenic population regularly for shifts in sensitivity to fungicides is crucial to mitigate the risk of fungicide resistance development. Resistance management strategies are being emphasized, such as rotating fungicides with distinct modes of action and integrating chemical control with biological agents or botanical extracts. Using fungicide combinations like Tebuconazole + Trifloxystrobin, which have dual modes of action, also reduces the likelihood of resistance development compared to single-mode fungicides. Furthermore, field trials and monitoring programs are in place to assess resistance trends and ensure the long-term efficacy of the applied treatments.

Pomegranate is rich in minerals like calcium, iron, and vitamins and is used in the pharmaceutical industry. It is also a medicinal crop to treat diabetes, cancer, gastric inflammation, and heart and kidney disease. Anthracnose is the most devastating disease affecting the fruits and leaves in field and storage conditions. Defoliation, flower and fruit dropping, decaying of fruits, and qualitative and quantitative loss of fruits are the significant effects in pomegranate due to the pathogen *C. gloeosporioides*. Management studies in field trials indicated that foliar spraying of carbendazim + mancozeb (0.3 %) followed by propiconazole (0.1 %) reduced the fruit rot, showing a 96 per cent reduction of the disease, moreover exhibiting 6.35 tonnes/ha, which is a 2.5 times higher than the yield of control (20). These consistent results suggest that azole-based fungicides (eg. Tebuconazole + Trifloxystrobin) are reliable for effective pomegranate anthracnose control (16).

The observed performance of neem seed kernel extract and *Bacillus subtilis* as alternative treatments also highlight the potential for integrating sustainable practices into disease management programs. Further studies focusing on the comparative efficacy of these treatments under varying environmental and agronomic conditions would strengthen recommendations for farmers seeking eco-friendly solutions. To manage fruit diseases, selecting low-risk and environmentally safe fungicides is essential. Alcoholic, aqueous and oil extracts of neem seeds were prepared and evaluated under different concentrations (1000, 2000, 4000, 8000 and 10,000 µl/L) *in vitro* to control the mycelium of *C. nymphaea*, which caused anthracnose in strawberries. The alcoholic extract (10,000 µl/L) achieved 80 % inhibition of *C. nymphaeae* mycelial growth (17). Fruit rot caused by *Phoma lingam* is an essential disease in custard apples, changing the fruits into coal-tar balls. Among the various botanical extracts tested against *P. lingam*, neem leaf extract (5 %) reduced mycelium growth by 76.3 %, followed by *Cymbopogon citratus* (Lemon grass), the leaf extract with 71 % reduction. Under field conditions, spraying the combination of Bavistin + Azadirachtin extracts reduced fruit infection by 72%, followed by Bavistin + *Cymbopogon* plant extract (18). In chemical management, two systemic fungicides (Fenamidone and Azoxystrobin) and four contact fungicides (Zineb, Propineb, Captan and Copper Oxychloride) were evaluated against the pathogen *Colletotrichum gloeosporioides* causing dieback in citrus at the recommended concentrations *in vitro* based on poisoned food technique. Among these, the contact fungicide, *viz*., copper oxychloride, showed 100 % inhibition of the pathogen at 0.5 % concentration, followed by Captan (0.5 %), which inhibited mycelial growth (19).

Mango productivity is highly affected by anthracnose. Even though organic sulfur fungicides (Zineb and Mancozeb) and heterocyclic nitrogenous compounds (Captan) exhibited sufficient control over the disease, they also showed some phytotoxic effects. Strobilurins were introduced to alternate the above-contact fungicides. These fungicides showed broad-spectrum activity against Ascomycetes and Basidiomycetes fungi. Under laboratory conditions, this fungicide ultimately arrested the mycelial growth of *C. gloeosporioides* at 1, 2 and 4 % concentrations. In the field experiment, spraying Azoxystrobin (1,2 and 4 ml/L concentrations) also showed a 100 % reduction of anthracnose. Trees treated with this fungicide produced a maximum number of fruits (40 kg/tree) compared to control (13.8 kg/tree). This fungicide didn't exhibit any phytotoxic effect (20).

Anthracnose in walnuts is a significant disease influencing this crop's yield, quality and marketability. *Colletotrichum* species complex are the most harmful and economically deleterious disease. The bacterial biocontrol agent *Bacillus velezensis* CE 100 was used to control the above pathogen. Field application of *B.velezensis* CE 100 culture broth recorded a 9-fold decrease in anthracnose disease compared to control. Soil inoculation of this culture improved the root development, nutrient uptake, chlorophyll content and total biomass. The crude enzyme extracted from this bacterium, which possesses chitinase, protease, and glucanase enzymes, also degraded the pathogen's cell wall. These biocontrol agents and endophytes have to be promoted to contain the pathogens of fruits. In onion, *C. gloeosporioides* is also the major pathogen referred to by the disease as twister blight. Around 40 endophytes were isolated from the root and stem portion of onion. Only two isolates showed maximum antifungal activity in the dual plate technique and were also confirmed as non-pathogenic on onion crops based on the hypersensitive test. They were also non-pathogenic in mammals based on the confirmation through hemolysis test (21–22).

Conclusion

Our study revealed that *C. gloeosporioides* and *C. siamense* are the primary pathogens responsible for leaf spot and fruit rot in custard apples. The combined formulation of Tebuconazole and Trifloxystrobin exhibited high efficacy in reducing disease incidence by 63.76 % and increasing yield to 15.50 kg/tree. Environmentally safer botanicals, such as neem seed kernel extract (5 %) and thyme oil, showed 51.20 % and 45.30 % reductions in fruit rot incidence, respectively, offering eco-friendly alternatives that aid in resistance management. These findings confirmed the importance of integrating chemical and biological approaches under the Integrated Disease Management (IDM) framework for long-term sustainability. Future research should focus on field-scale validation of these treatments and monitoring potential resistance development in pathogen populations.

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Authors' contributions

AR and CSRK initiated the research work and designed the experiments. AR carried out the basic laboratory experiments and wrote and upgraded the text and diagrams. CR helped correct the manuscript and improve the language. PB, as a scheme officer in ICAR -AICRP-AZF, is involved in maintaining experimental trials. All the authors have gone through this research paper and accepted it.

Compliance with ethical standards

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While writing this article, the authors referred to the software Grammarly to improve the language and readability. Based on this, the authors refined and upgraded this paper. The authors are highly responsible for the content and concepts of this research paper.

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