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New report of *Colletotrichum fragariae* Brooks causing tip rot and hardening of fruit in pomegranate (*Punica granatum* L.) and their management

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Abstract

Fruit hardening caused by *Colletotrichum fragariae* Brooks is a serious fungal disease of pomegranate. Survey revealed that, the maximum percent disease index (PDI) of tip rot and fruit hardening was observed in Arasikere (33.34 %), Tiptur (30.36 %) and Kanakapura (28.17 %) and other pomegranate cultivating areas. The pathogen first infecting calyx end or middle portion of the fruit and produces light yellow spots on the fruit. Initially the disease was appeared as brown discoloration at calyx end and later it covered entire fruit and became hard. Necrotic patches on the surface of the fruits and aril discoloration were observed. The pathogen identified based on morphological and molecular characterization as *Colletotrichum fragariae*. The colony and morphological characters of the *C. fragariae* on potato dextrose agar medium produced white coloured mycelium, hyaline conidiophore with single celled hyaline conidia which is straight cylindrical, tapered end at one side and round at another end, with one to two oil globules present either end of the conidia, further confirmation was done by molecular characterization by using 18S rDNA region sequencing. On the basis of morphological, molecular and cultural characters, the fungus was identified as *C. fragariae* (MH251944). Pathogenicity of the fungus on pomegranate flowers and fruits were proved by inoculating conidial suspension of the pathogen and symptoms on both flowers and fruits and expressed within seven days after inoculation. Complete inhibition of fungal growth of *C. fragariae* was observed in fungicides, Mancozeb, Tebuconazole, Propiconazole, Hexaconazole, Difenconazole and Propiconazole + Difenoconazole at all the concentrations tested. The best fungicide is Difenoconazole (0.1 %) and Propiconazole + Difenoconazole (0.1 %) against fruit hardening pathogen in pomegranate.

Keywords : Pomegranate, Tip rot, Fruit hardening and *Colletotrichum fragariae*

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Introduction

Pomegranate (*Punica granatum* L.) is an important fruit crop grown in tropical and subtropical regions of India and is commonly known as Dalimbe, Anar, and Matulum. In recent years, pomegranate has threatened by major fungal diseases on fruits, tip rot and fruit hardening of pomegranate caused by *Colletotrichum fragariae* is the most threatening disease of pomegranate and causes 30 to 50 per cent fruit loss. The pathogen first infecting calyx end or middle portion of the fruit and produces light yellow spots on the fruit. Initially the disease was appeared as brown discoloration at calyx end and later it covered entire fruit and became hard. Necrotic patches on the surface of the fruits and aril discoloration were observed and finally the fruit dried due to infection. Successful cultivation of pomegranate in recent years has threatened by major fungal pathogens viz., *Aspergillus* fruit rot, *Pencillium* fruit rot, *Cercospora* fruit rot, Anthracnose, Bacterial blight. Among the various fungal diseases, fruit rot caused by *Colletotrichum fragariae* is the most threatening

disease of pomegranate and fruit loss due to this disease ranges from 30 to 50%. However, there was a report by Howard (1972) on strawberry in Florida that this pathogen also cause's fruit rot.

Fruit hardening of pomegranate is one of the contributing factors for low productivity and this disease is spread at faster rate after heavy rainfall. This study made to study the fruit tip rot and fruit hardening of pomegranate with suitable fungicides for the management of this disease.

Materials and Methods

The intensive roving survey was conducted during 2017-19 to know the incidence and severity of fruit hardening disease of pomegranate in Kanakapura, Pavagada, Tiptur, Arasikere, Tumakuru, Hassan, Chikkaballapur, Kolar and Chithradurga and other districts in Karnataka. In field, plants were selected in zigzag manner. The symptoms and severity of fruit rot disease of pomegranate was recorded and data converted in to Percent disease index (PDI) Wheeler (1969).

Infected pomegranate fruits were collected from different locations viz., Kanakapura, Pavagada, Tiptur, Arasikere, Tumakuru, Hassan, Chikkaballapur, Kolar and Chithradurga during survey and the isolation of the fungus was done by following standard tissue isolation technique. The infected portions along with some healthy parts were cut and surface sterilized using 0.1% mercuric chloride solution for 15 sec. These bits were thoroughly washed in sterile distilled water to remove the traces of mercuric chloride, if any and then aseptically transferred to sterile potato dextrose agar (PDA) petriplates and incubated at room temperature (27±1°C) and observed periodically for fungal growth and sporulation. Colonies, which developed from the bits, were identified by microscopic observation by taking mycelial and spore character as means for identifying the pathogen. After identification they were transferred to new PDA slants and incubated at 27±1°C for further use. The morphological characters of the fungus such as mycelial and cultural characters, length and breadth of conidia, fruiting body were studied by using a microscope.

The mycelium collected from the liquid cultures in potato dextrose broth after 7-8 days of incubation was filtered through Whatman No.40 filter paper. The mycelia were then dried completely by pressing in between folds of pre-autoclaved filter papers. The DNA extraction method was standardized and certain steps were optimized to produce good concentration of DNA using plant DNA isolation kit (C-TAB method). The quality and quantity of DNA was analyzed by running 2 µl of sample mixed with 2 µl of 10x loading dye in 1.5% agarose gel. The DNA from the sample prepared produced clear sharp bands in 1.5% agarose gel indicating good quality of the DNA. The DNA was quantified by comparing with the 1 kb size marker (Genei Pvt. Ltd. Bengaluru). The gel was observed under UV light and documented using gel documentation unit. The ribosomal DNA (rDNA) unit contains genetic and non-genetic or spacer region. Each repeat unit consists of a copy of 18s rDNA. The 18s rDNA have been employed to analyze evolutionary events because it is highly conserve, whereas ITS rDNA is more variable. Hence, it has been used for investigating the species level relationships. The primers for amplification were custom synthesized at Bengaluru Genie Pvt. Ltd. and supplied as lyophilized products of desalted oligos. Agarose gel electrophoresis was performed to resolve the amplified product using 1.5% agarose in IX TBE (Tris Borate EDTA) buffer, 0.5 ug ml^{-1} of using Ethidium bromide and loading buffer (0.25% Bromophenol Blue in 40% sucrose). Four µl of the loading dye was added to 20 µl of PCR product and

loaded to the agarose gel. Electrophoresis was carried at 65 V for 1.5 h. The gel was observed under UV light and documented using gel documentation unit. The 18s rDNA region was sequenced to confirm organism. The PCR product was sequenced using forward and reverse primers at Sakhala enterprise Bengaluru. Homology search done using BLAST algorithm available at the VA3T.ncbi.nlm.nih.gov. Multiple alignments for homology search performed using the Clustral Walgorithm software and the phylogenetic tree was constructed.

The healthy young fruits were selected from the pomegranate plant to prove the pathogenecity; fruits were washed thoroughly with tap water, and wiped using moist cotton swab. The inoculum suspension from 12 days old culture was prepared in potato dextrose broth with 1×10^6 spores /ml were used for spraying. Branches were covered with polythene bags for 48 h. to ensure successful penetration of the pathogen into the tissue. Similarly control plants were sprayed with sterile distilled water for comparison. The polythene bags were removed after 2 days and observation was made regularly for the appearance and development of symptoms. After appearance of disease symptoms of disease symptoms re-isolation were made from the diseased tissue of artificially infected plants. The culture thus obtained was compared with original culture to confirm the identity of the fungus and subsequent confirmation of Koch's postulates.

The efficacy of fungicides was tested against *C. fragariae* on potato dextrose agar media by using poisoned food technique under in vitro condition. The fungicides were tried at 250, 500 and 1000 ppm concentrations; the required concentration of chemicals was prepared and incorporated into sterilized and cooled potato dextrose agar. Twenty ml of cooled medium was poured into 90 mm sterilized Petri dishes and all plates were inoculated with actively growing five mm mycelial disc of pathogen separately. Three replication were maintained for each treatment. These plates were incubated at 25°C for seven days and colony diameter was recorded. Per cent inhibition of mycelial growth over control was calculated by using the formula of Vincent (1947). Effective fungicides were further evaluted under field conditions. An experiment was conducted in the farmer's orchard located at Karadihalli of Hassan district during 2017-2018 in relation to manage the fruit rot of pomegranate. The variety, Bhagwa was used and sprayed with different fungicides with recommended concentration of fungicides. The effect of different fungicides on fruit rot disease incidence was recorded based on 0-4 scale. Scale 0: No infection with zero per cent of infection: scale1: 1-25% tip of the fruit infection: scale 2: 26 to 50% fruit (Tip of the fruits and center of portion of the fruit) infection, scale3: 51 - 75% fruit infection; scale 4: above 75% fruit infection with drying of fruits.

Results and Discussion

Roving survey was conducted during 2017 - 2018 to assess the severity of fruit tip rot fruit hardening of pomegranate in Hassan, Tumakuru, Chitradurga, Ramanagara and Kolar and other pomegranate growing areas. Totally 59 fields of 10 taluks and 5 districts were surveyed during 2017-18. Per cent disease severity of fruit rot ranged from 13.45 to 38.97. Highest per cent disease severity (38.97) was recorded in Ambae bahar in Shashivala village of Tiptur taluk followed by Yogenahalli (38.22 PDI) of Sira taluk and Rampura (37.55 PDI) of Arasikere taluk. The lowest per cent disease severity (13.45) was recorded in Hosalli of Kolar taluk. Mean severity of fruit rot disease in different districts of southern Karnataka during 2017-18 was recorded. The maximum disease severity of 30.55% was recorded in

Tumakuru district followed by Hassan (29.59%). Lowest fruit rot disease severity of 20.54% was observed in Kolar district. Among the three bahar, maximum fruit rot disease severity was recorded in Mrig bahar (30.22%) and moderate in Ambae bahar (23.73 %). The least per cent severity was 23.57.

The pathogen, *C. fragariae* infection occurs during flowering and fruit formation stage. The pathogen *C. fragariae* infecting on flowers, the disease initially appears as a black colour discolouration on the calyx end of the flowers and later it spreads to whole flowers and finally flowers turns to brown to black and becomes hard and dry (Fig.-1). The pathogen *C. fragariae* infecting major parts of the fruits starting from fruit setting to maturity stage of the fruit. The pathogen first infecting either on the tip or middle portion of the fruit with light yellow spots on the fruit (Fig.-1). the disease first appear as brown discoloration on calyx end of the fruit and in advanced stage it covers entire fruit and become hard and necrotic patches on the surface of the fruits. When such diseased fruits were cut open, aril turns to brown to dark brown discoloration (Fig.-1). Similar descriptions about the symptoms were given on strawberry by ZhaoXing *et al.* (2016). Earlier, Howard (1972) described the typical symptoms of fruit rot of strawberry caused by *Colletotrichum fragariae.*

The pathogen was isolated from infected parts by satndard tissue isolation technique. Identification of the fungus was carried based on the morphological characters and found that, the fungus produced septate mycelium, conidiophores arising singly or closely packed together in rows. Conidiophores were single celled, hyaline and aseptate with one or several conidial scars. *C. fragariae* produced longer conidia with high proportion of tapered end and beared conidia on setae. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate and one to two oil globules were observed in the conidium. These characters of the fungus is similar to the one described by Smith and Barbara Jones (1985) who reported the morphology of *C. fragariae* on PDA medium, which was single celled, hyaline and aseptate, tapered cylindrical, to one end and rounded on other end. Fungus was isolated from infected pomegranate fruits/flowers and pure culture was obtained by single spore isolation and such culture was used for pathogenecity test and molecular identification. The pure culture of *C. fragariae* was inoculated to the pomegranate flowers and fruits (106 conidia/ml). Initial symptoms viz., small patch of black colour rotting at calyx end of the flowers was visible after five days of inoculation and on sixth day rottening patches were observed on whole flower and became hard, while on seventh day rottened flowers were dropped. To prove the Koch's postulates, the pathogen was re-isolated from the infected flowers on PDA medium and the reisolated culture was similar to the original culture. These results were similar to the symptoms that were described by El Gali (2008), who described the symptoms of rotting of the flowers and fruits in strawberry and dropping of rotted flowers and the isolated fungus from infected tissues was identified as *Colletotrichum fragariae*. The identified pathogen was confirmed by pathogenicity test.

The conidia of *C. fragariae* were obtained from infected fruit and culture was measured and compared with respect of their spore morphology. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with tapered ends and one to two oil globules present in conidia. Conidia on the culture media formed with grayish slimy mass. They were rarely found in aggregates. The conidia collected from potato dextrose agar measured under 40X microscopic field, the length of conidia varied from 1.04-2.39 µm and

Fig. -1. Symptoms on flowers and fruits along with colonies of *Colletotrichum fragariae*

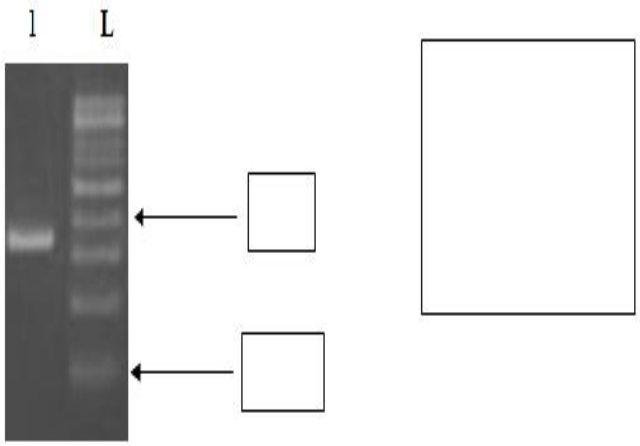


Fig.2. PCR amplification of 18S rDNA fragment from sample The size of PCR amplified product is ~1.8kb

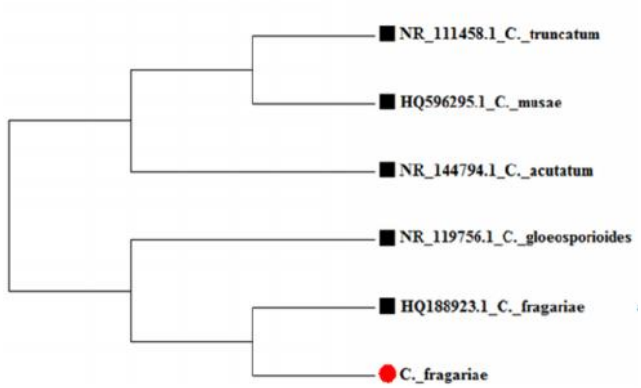
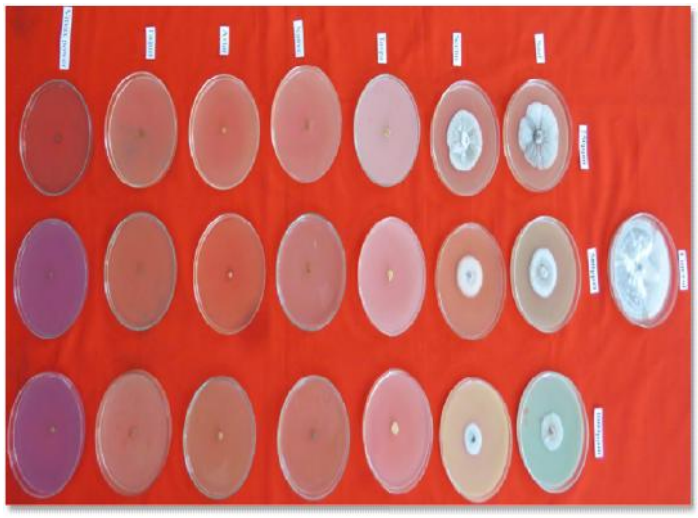


Fig.-3. Phylogenetic tree construction using Mega7 software

breadth varied from 0.20-0.36 µm. Similar study was carried out by Smith and Barbara Jones (1985). They reported that fungus produced tapered end cylindrical conidia, one end of the conidia was tapered and other end was rounded. The volume of the *C. fragariae* conidia was calculated by the formula V = 0.25TTW2 which combines the volumes of a cone, a cylinder and 1/2 a sphere, roughly the shape of the *C. fragariae* conidia.

T1: Carbendazim, T2: Tebuconazole, T3: Propiconazole T4: Difenoconazole, T5:Hexaconazole



T1: Carbendazim + Mancozeb T2: Fenamidon + Mancozeb T3: Propiconazole + Difenoconazole T4: Tebuconazole + Trifloxystrobin, T5: Hexaconazole + Zineb, T6: Hexaconazole + Captan, T7: Carboxin + Thiram

Fig.- 4. *in vitro* Evaluation of fungicides against *C. fragrariae*

Table – 1. *In vitro* evaluation of systemic and non-systemic fungicide against C. fragariae

Sl. No.	Fngicides	Percent inhibition over control Concentration			
		250ppm	500ppm	1000ppm	Mean
1.	Carbendazim	33.64 (35.46)*	41.04 (39.85)	49.43 (44.68)	41.37 (40.04)
2.	Tebuconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90)
3.	Propiconazole	100(90.00)	100 (90.00)	100 (90.00)	100 (90)
4.	Hexaconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90)
5.	Difenaconazole	100 (90.00)	100 (90.00)	100 (90.00)	100 (90)
6.	Chlorothalonil	50.03 (45.02)	42.58 (40.74)	69.62 (56.56)	54.08 (47.35)
7.	Capton	38.68 (38.47)	67.00 (54.83)	80.70 (63.95)	62.13 (52.03)
8.	Mancozeb	40.16 (39.33)	34.28 (35.84)	40.47 (39.51)	38.30 (38.24)
	Mean	**70.31** (64.79)	**73.11** (66.41)	**80.03** (70.59)	**74.48** (59.67)
			Fungicides (F)	**Concentration (C)**	**Interactions (F×C)**
	S. Em. ±		**0.49**	**0.30**	**0.85**
	C.D. @ 1%		**1.87**	**1.14**	**3.23**

* The values in the parenthesis is arcsine transformed

The DNA of the fungus causing fruit hardening was isolated and the 18s rDNA region of the fungal genome was amplified in a PCR using 18s rDNA primer. The amplified region was typically 1.8 kb in length. The amplified product was checked by electrophoresis in 1.5% agarose gel (Plate 2). The amplified region was sequenced and compared using bioinformatics tools like NCBI (National Centre for Bioinformatics) BLAST programme. BLAST search has shown 99% similarity of the query sequence with *Colletotrichum fragariae*. Based on phylogenetic study it was confirmed that *C. fragariae* was the causal agent of fruit hardening of pomegranate. 18s rDNA sequence of *Colletotrichum fragariae* was deposited in NCBI with Gen Bank accession number (MH251944). The 18s rDNA region has been extensively used for the species-level discrimination of fungal species. Universal-primed polymerase chain reaction (UP-PCR) fingerprinting, coupled with the restriction of 18s rDNA regions helps in the identification of five groups of *Colletotrichum i*.e., *C. fragariae*, *C. gloeosporioides*, *C. musae*, *C. truncatum*, and *C. acutatum*. Phylogenetic tree was constructed using Mega6 software. The cluster analysis showed that the *C. fragariae* [MH251944] had closer genetic relationship with *C. fragariae* [HQ188923] causing fruit rot of strawberry, and they were clustered in the same branch and *C. gloeosporioides* [NR119756] (Fig.- 3). Based on morphological characteristics, molecular data (rDNA region and sequence analysis) and pathogenicity test, it was suggested that the pathogen *C. fragariae* was confirmed to be responsible for fruit hardening of pomegranate. Villanueva *et. al.*, (2005) reported the anthracnose of cherimoya (*Annona cherimola*) fruits. In characterization and identification of *Colletotrichum fragariae* on cherimoya fruits, the ITS1 region sequence of ribosomal DNA showed 99.8% identity similarity index with *C. fragariae* in Mexico. Dai Qi *et. al.* (2013) reported the anthracnose of Raspberry. Morphological and molecular identification of the pathogens *Colletotrichum fragariae* and *C. gloeo sporioides* sequence analysis of the internal transcribed spacer region of the ribosomal DNA was also done. A specific fragment was amplified using fungus universal primers ITS1 and ITS4 by the PCR method.

Availability of different mode of action of fungicides necessitates their evaluation under *in-vitro* and in vivo condition against *C. fragariae*. Complete inhibition of growth of *C. fragariae* was recorded in systemic fungicides viz., Tebuconazole, Propiconazole, Hexaconazole and difenoconazole at 250,500 and 1000 ppm concentrations and among the non-systemic fungicides least per cent inhibition was noticed in mancozeb (41.14 %). However, maximum per cent inhibition of mycelial growth was at 1000 ppm concentration in all systemic fungicides viz., tebuconazole, propiconazole, hexaconazole and difenoconazole showed hundred per cent inhibition of mycelial growth. Whereas least inhibition per cent was in carbendazim (41.37%). Among non-systemic fungicide captan showed maximum (67.00 %) mycelial growth inhibition followed by chlorothalonil (42.58 %) and mancozeb (41.48 %). Among non-systemic and systemic fungicides, highest inhibition per cent was recorded at 500 and 1000 ppm concentration (Table - 1). Smith and Black (1993) conducted in-vitro evaluation of fungicides against *C. fragariae* causal agent of fruit rot strawberry and results revealed that they evaluated 28 fungicides at different concentration and recorded the colony diameter of *C. fragariae* was reduced at concentration of 50 ppm. Systemic fungicides like difenoconazole, flusilazole, benomyl, pro piconazole, nuarimol, fenarimol and bitertanol severely limited the growth of pathogen at 0.5 ppm and

Table – 2. *In vitro* evaluation of combi product fungicide against *C. fragariae*

Sl. No.	Fungicides Common name	Percent inhibition Concentration (PPM) 250	500	1000	Mean
1.	Tebuconazole 50 % WG + Trifloxystrobin 25 % WG	100 (90.00)*	100 (90.00)	100 (90.00)	100 (90.00)
2.	Hexaconazole 5 % + Captan 70 % WP	100 (90.00)	100 (90.00)	100 (90.00)	100(90.00)
3.	Fenamidon 10 % + Mancozeb 50 % WG	58.50 (49.90)	63.72 (52.97)	73.35 (58.92)	65.19 (53.93)
4.	Carboxin 37.5 % + Thiram 7.5 % WP	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
5.	Propiconazole13.9 % + Difenconazole13.9 % EC	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
6.	Carbendazim 12 % + Mancozeb 63 % WP	45.93 (42.67)	55.98 (48.44)	68.68 (55.97)	56.86 (49.02)
7.	Hexaconazole 4 % + Zineb 68 % WP	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
	Mean	86.34 (77.51)	88.52 (78.77)	91.72 (80.70)	88.86 (78.99)
		Fungicides (F)	**Concentration (C)**	**Interactions (F×C)**	
	S. Em. ±	**0.33**	**0.20**	**0.57**	
	C.D. @ 1%	**1.23**	**0.75**	**2.14**	

*The values in the parenthesis is arcsine transformed

higher concentration and among non-systemic fungicides like mancozeb, maneb, thiram, dicloran, iprodione, triforine, chlorothalonil and triadimefon reduced colony diameter by 20%. Among non-systemic fungicides, chlorothalonil was showed highest percent of inhibition (70.74 %) at 0.3 per cent concentration and least inhibition was recorded in dithane M-45 at 0.3% (50.37 %). Devamma *et. al.* (2012) evaluated fungicides against *C. gloeosporioides* that causes mango anthracnose and found thiophanate-methyl (100 %) and the non-systemic fungicide, mancozeb (100 %) proved to be effective in inhibiting the mycelial growth of the highly virulent pathogen at 50 ppm and 500 ppm concentrations respectively.

The inhibition of mycelial growth of *C. fragariae* at three different concentrations (250, 500 and 1000 ppm) were evaluated. Among seven combi products fungicides tested, hundred per cent inhibition of *C. fragariae* was recorded in Tebuconazole + Trifloxystrobin, carboxin + thiram, hexaconazole + captan, propiconazole + difenconazole and hexaconazole + zineb treated plates at all concentration (250, 500, 1000 ppm). Whereas least inhibition of mycelial growth was observed in carbendazim + mancozeb with 56.86% inhibition. Among the three tested concentrations, 1000 ppm concentration (91.72 %) was on par with 500 ppm (88.52 %) concentration, followed by 250 ppm (86.34 %). At 500 ppm concentration, tebuconazole + trifloxystrobin, carboxin + thiram, hexaconazole + captan, propiconazole + difenconazole and hexaconazole + zineb showed hundred per cent mycelial growth inhibition of *C. fragariae* followed by fenamidon+mancozeb (63.72 %) and carbendazim + mancozeb (55.98 %). Similarly, at 1000 ppm concentration, tebuconazole + trifloxystrobin, carboxin + thiram, hexaconazole + captan, propiconazole

Table – 3. Field evaluation of fungicides against fruit hardening disease on pomegranate

Treatments		Before spray	PDI on fruits after 1st to 6th spray							AUDPC values
			After 1st spray	After 2nd spray	After 3rd spray	After 4th spray	After 5th spray	After 6th spray	Percent disease reduction over control	
T_1	Propiconazole	22.46 (28.30) *	21.11 (27.36)	18.91 (25.78)	17.80 (24.96)	16.41 (23.90)	14.88 (22.69)	12.29 (20.53)	58.38	745.40
T_2	Fenamidon + Mancozeb	22.54 (28.35)	21.00 (27.28)	18.85 (25.74)	17.75 (24.92)	15.84 (23.46)	14.53 (22.41)	12.32 (19.81)	58.27	761.79
T_3	Difenoconazole	23.06 (28.71)	22.22 (28.13)	18.68 (25.61)	15.89 (23.50)	14.29 (22.22)	11.86 (20.15)	10.16 (18.6)	65.59	696.85
T_4	Tebuconazole	22.63 (28.41)	21.58 (27.69)	18.96 (25.82)	17.97 (25.08)	16.27 (23.80)	15.46 (23.16)	13.63 (21.67)	53.84	758.58
T_5	Mancozeb	24.00 (29.34)	23.27 (28.85)	22.53 (28.34)	21.30 (27.49)	19.75 (26.39)	18.38 (25.39)	17.22 (24.52)	41.68	880.96
T_6	Hexaconazole	23.67 (29.11)	22.98 (28.65)	21.99 (27.97)	20.46 (26.90)	18.27 (25.31)	15.78 (23.41)	16.12 (23.68)	45.41	835.61
T_7	Propiconazole+ Difenoconazole	22.22 (28.13)	21.92 (27.92)	20.48 (26.91)	18.09 (25.18)	16.51 (23.98)	14.58 (22.45)	11.48 (20.51)	61.12	734.79
T_8	Control	23.92 (29.29)	24.51 (29.68)	25.96 (30.64)	26.18 (30.78)	27.53 (31.65)	28.15 (32.05)	29.53 (32.92)	--	1113.12
SEm ±		**0.74**	**0.71**	**0.90**	**0.570**	**0.59**	**0.61**	**0.53**	--	
CD at (5%)		**2.25**	**2.15**	**2.74**	**1.73**	**1.80**	**1.87**	**1.61**		

*The values in the parenthesis is arcsine transformed

+ difenconazole, showed hundred per cent mycelial growth inhibition of *C. fragariae* followed by fenamidon + mancozeb (73.35 %) and carbendazim + mancozeb (68.68 %) (Table - 2). A similar inhibition acivity of carbendazim + mancozeb was also reported by Patel (2009).

Field experiment was conducted to evaluate the efficacy of fungicides against fruit hardening of pomegranate during 2017-18 at Karadihalli village of Arasikere taluk, Hassan district. The effect of different fungicides on fruit hardening disease incidence was recorded using 0-4 scale. The experiment was conducted during Hasth bahar cropping season during 2017 - 18, with seven treatments and one untreated which served as control. Totally 6 sprays were sprayed at an interval of 7 days. The observation on pomegranate fruit hardening was recorded at 15 days interval. Further, these observations were converted into per cent disease index (PDI) (Wheeler, 1969). The maximum PDI on fruit was noticed in untreated control (29.53 PDI) after sixth spray (Table 3). The experiment revealed that the severity of the disease on fruits before

the treatment application was non-significant and almost uniform in the plots and significant differences among the treatments were observed after second spray. After first spray, the disease was relatively consistent and all the treatments remained on par with each other. On the contrary after 2nd, 3rd, 4th, 5th and 6th spray treatment differed significantly interms of disease severity. After 2nd spray, the minimum PDI (18.68) was recorded in difenoconazole treatment which was on par with propiconazole, fenamidon + mancozeb and tebuconazole respectively. Maximum PDI was recorded in control. After 4th spray, the difenoconazole recorded the lowest PDI (14.29) and remained on par with fenamidon + mancozeb (15.84), tebuconazole (16.27) and propiconazole (16.41). Whereas the manxozeb was least (19.75) effective. After 6th spray, the difenoconazole showed significantly good result (10.16) as compared with all the treatments followed by fenamicdion + mancozeb (11.48) which was on par with propiconazole + difenoconazole (12.27) and propiconazole (12.29). Least reduction was observed in mancozeb (17.22). Untreated had increased in PDI (29.53). Further per cent disease reduction over control (PDC) was calculated for all the treatments. Among seven treatments, the highest PDC was observed in difeoconazole (65.59) followed by propiconazole + difenoconazole (61.12) and propiconazole (58.44). Least reduction was observed in mancozeb (41.68) (Table 3). Jamadar *et.al.* (1998) reported that the combi product like Mancozeb 0.2 % + Carbendazim 0.05 per cent was more effective in controlling fruit spot incidence over control followed by Bordeaux mixture, reduced the disease by more than 88% against control. The results obtained were in agreement with the Jayalakshmi (2010) who reported that the combi product (Carbendazim + Mancozeb) at 0.3 per cent (PDI of 0.83) and propiconazole at 0.1 per cent (1.20) controlled the anthracnose very effectively in the orchard. Spraying of 0.1 per cent Difenoconazole against *C. gloeosporioides* causing anthracnose of pomegranate showed least per cent disease intensity and maximum percent disease control as reported by Navale *et. al.* (2009). Patel (2009) reported that, under field condition Carbendazim sprayed fruits showed highest per cent disease control over unsprayed fruits and Propineb showed lowest percent disease control against C. gloeosporioides of pomegranate.

Pomagranate fruit hardening is due to infection of *Colletotrichum fragariae* is become severe during heavy rainfall period and cause brown discolouration at the tip of the fruits and extends to fruit, it becomes intially light yellow colour and later changes to dark brown and ultimately drying of fruits. It can be effectively managed by spraying difenoconazole and propiconazole + difenoconazole at 0.1 per cent concentration.

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