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Isolate variations in *Colletotrichum gloeosporioides* Penz. infecting major fruit crops in Khandesh region of Maharashtra

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Abstract
Major fruit crops were tested against twelve isolates of *Colletotrichum gloeosporioides* under controlled conditions. Inoculated fruits were assessed on the basis of virulence of the isolates and reaction of a particular variety was expressed in Virulence Index (VI). There was considerable variation in virulence and susceptibility within isolates and fruit crops. Isolates CgSD – 4, CgPD -1 and CgGJ 11 was found to be highly virulent (VI – 5.58, 5.03 and 4.46), CgBN – 9 as extremely less virulent (VI-1.96), and other isolates were highly virulent, moderately virulent, less virulent irrespective of fruit crops. Fruit crop guava were found to be extremely succeptible as displayed maximum mean of VI – 5.58, respectively irrespective of isolates. Resistant fruit crops pomegranate and banana showed uniform resistant reaction against twelve isolates of the pathogen. The interaction between isolates X fruit crops was also statistically significant. Highest VI (11.99) was recorded in the interaction between CgSD – 4 X sweet orange.

Keywords: *Colletotrichum gloeosporioides*, isolate, virulence index, fruit crops

Introduction
Fruit crops is extensively cultivated around the Mediterranean and other parts of world including India. In India the area under fruit crops is 64.8 lakh ha by the end of 2017-18. The major fruit crops which exhibited considerable increase in their acreage are pomegranate (2.09 lakh ha), sweet orange (1.85 lakh ha), banana (8.58 lakh ha), guava (2.62 lakh ha) area followed by mango, sapota, grape, cashew (Annonymous, 2018) [1]. Maharashtra is the leading producer of horticultural fruit crop as compared to other state. In Maharashtra area under fruit crops is 7.63 lakh ha and production 10378.43 MT in 2016-17. The estimated area under pomegranate cultivation in Maharashtra accounts 136.75 thousand ha area and 1578.04 MT production. Under sweet orange, with an area of 54.89 thousand hectares and production was 656.89 MT. Banana had 74.68 thousand hectares of area and production of 3078.73 MT. Guava fruit crop had 12.49 thousand hectares area and production was 140.86 MT (Annonymous, 2018) [1]. *Colletotrichum gloeosporioides* is responsible to cause localized epidemics in fruit plantations as well as it is the predominant post-harvest pathogen of fruits worldwide. In India anthracnose caused by *C. gloeosporioides* is the most important disease of pomegranate, sweet orange, banana and guava fruit crops. The severity of anthracnose has also been reported by several workers (Raghuvanshi *et al*. 2005; Mandhare *et al*. 1996; Kale and Raut (1985) [8, 5, 3]. The average losses caused by anthracnose in pomegranate was 20-30%, in sweet orange and banana 30-40% and in guava 25- 40%. The disease is more severe in the fruiting stage of *Mrig bahar* cropping season (Rainy season fruiting) which leads to post harvest rotting of fruits. Therefore the study was carried out to identify host resistance against the pathogen; fruits crops of pomegranate, sweet orange, banana and guava were tested against twelve isolates of *C. gloeosporioides* recovered from endemic area of Maharashtra.

Materials and Methods
Fruits showing typical anthracnose symptoms were collected from different fruit crops in different district of Khandesh region Maharashtra State for getting isolates of *C. gloeosporioides*. Monoconidial cultures of twelve distinct isolates were subjected to pathogenicity test separately for each isolate. Large, uniform sized healthy fruits of different varieties having uniform maturity were obtained from disease free garden of the College of

Agriculture, Dhule. Fruits were washed and then deposited in 0.1% $HgCl_2$ for 120 seconds for surface sterilization followed by washing with sterilized water and drying the surface with sterilized blotting paper. Such fruits were inoculated in a similar fashion as described in the pathogenicity test. Inoculated fruits were kept in the humid chamber for 10 days (28°C temperature and 90% RH). A set of uninoculated fruits of each fruit crops was also maintained for comparison. Intensity of disease was recorded ten days after inoculation. The infectivity of each isolate on different fruit crops was confirmed upon re-isolation from each fruit inoculated after 10 days.

The observations on latent period and severity of lesion development on fruits of pomegranate, sweet orange, banana and guava were recorded and average virulence index of each isolates on different fruits was determined. The data obtained were subjected to statistical analysis by Factorised Completely Randomised Design (FCRD). The virulence index (VI) was calculated by modifying the formula of Mathur *et al.* (2001) [6] to suit the requirement at present studies. The formula is based on the lesion diameter and the invasion of the pathogen. The invasion index (I) was rated as given in the Table.

Table: Ratings for invasion index (I)

Rating	Invasion index
1	Necrotic lesions developing superficially on the fruit peel/ skin.
2	Necrotic lesions comlpletely invading the peel/skin extending to the inner side of the peel and touching the pulp or aerials.
3	Invasion of the fungus in the pulp / aerials and causing it's discoloration.
4	Invasion deep seated reaching to the stone/heart of the fruit initiating the rotting symptoms.

VI = 3.14 X A/2 X I X L

Where, A = Aggresiveness (lesion diameter in mm)
I = Invasion index
L= Latent period in days (10)
3.14 = Area constant

The fruit crops and isolates rated respectively as resistant and extremely less virulent / avirulent (VI < 2.00), moderately resistant and less virulent (VI 2.01 – 2.50), moderately susceptible and moderately virulent (VI 2.51 – 3.00), susceptible and virulent (VI 3.01 – 4.00) and highly susceptible and highly virulent (VI 4.01 and more than).

Results and Discussion

C. gloeosporioides has an extensive host range particularly in sub-tropical areas. Cross inoculation experiments demonstrated variations in pathogenicity on their original host and isolatesother fruit crops. The level of host preference among *C. gloeosporioides* isolates from four subtropical fruit crops and the susceptibility of the hosts varied significantly. Out of twelve isolates of *C. gloeosporioides*, isolate CgSD 4, CgPD 1 and CgGJ 11 was found to be highly virulent (Mean VI – 5.58, 5.03 and 4.46) irrespective of fruit crops and was statistically superior over other isolates. Isolates CgGD 10,CgBD 7 and CgGN 12 was also virulent, isolates CgPJ 2, CgPN 3 and CgBJ 8 moderately virulent, isolates CgSN 6 and CgSJ 5 were found to be less virulent. The isolates having extremely low virulence index included isolates *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9) which recorded the lowest virulence index of 1.96.

Fruit crops expressed considerable variation in their susceptibility to *C. gloeosporioides* irrespective of isolates (Table 1). The fruit crops guava recorded highest mean of VI – 5.98 and sweet orange recorded mean of VI – 4.82 therefore these fruit crop were rated as highly succeptible. Pomegrante and banana fruit crops were resistant to *C. gloeosporioides* isolates (VI – 1.60 and 1.21). This study indicated that guava and sweet orange fruit crops are susceptible due to variation in virulence of *C. gloeosporioides* isolates. Similar results were reported by Quimio and Quimio (1975) [7] on differences in the degree of pathogenicity of guava on isolates of *C. gloeosporioides* on pomegranate, banana and sweet orange to cause infection on fruits of each of these hosts but there was variability in the degree of pathogenicity.

It was observed that the interaction between varieties and isolates was statistically significant. The highest VI (11.99) was recorded in the interaction between CgSD 4 X sweet orange and lowest VI (0.00) was recorded in interaction between CgSN 6 X pomegranate, CgBN 9 X pomegranate, CgGD 10 X pomegranate and CgGN 12 X pomegranate. This indicated that different isolates have specific fruit crops preference. *C. gloeosporioides* isolates produced larger lesions on their original host when compared with the alternate hosts (Wijeratnam *et al*., 2008) [11]. Smith (1990) [10] reported highly significant variable interaction between 15 cultivars of strawberry and 13 isolates of *C. fragari*, two isolates of *C. gloeosporioides* and five of *C. accutatum* isolates. Similar type of significant interaction between *C. gloeosporioides* isolates and custard apple varieties has been recorded (Gaikwad 2002) [2]. Simmonds (1965) [9] demonstrated that *C. gloeosporioides* isolates from fruit crops could readily cross-infect over a wide host range, however, isolates were most aggressive in infecting the host from which they were originally isolated.

Table 1: Variation in virulence index of *C. gloeosporioides* isolates and its cross infectivity potential on different fruits

Isolates	Pomegranate	Sweet orange	Banana	Guava	Mean
CgPD 1	5.81	6.97	0.63	6.70	5.03
CgPJ 2	2.58	1.98	0.75	6.13	2.86
CgPN 3	0.77	1.46	1.76	6.63	2.66
CgSD 4	1.28	11.99	2.49	6.57	5.58
CgSJ 5	1.61	1.70	0.00	6.00	2.33
CgSN 6	0.00	1.27	1.88	6.70	2.46
CgBD 7	2.92	5.51	1.06	5.93	3.86
CgBJ 8	1.79	1.74	1.23	5.63	2.60
CgBN 9	0.00	0.95	1.66	5.23	1.96
CgGD 10	0.00	10.17	0.69	5.13	4.00
CgGJ 11	2.44	8.73	1.36	5.33	4.46

CgGN 12	0.00	5.42	0.98	5.70	3.02
Mean	1.60	4.82	1.21	5.98	3.40
				SE ±	CD @ 5%
Treatment				0.02	0.05
Isolates				0.01	0.03
Treatment x Isolates				0.06	0.18

Where, Treatment means Fruit crops

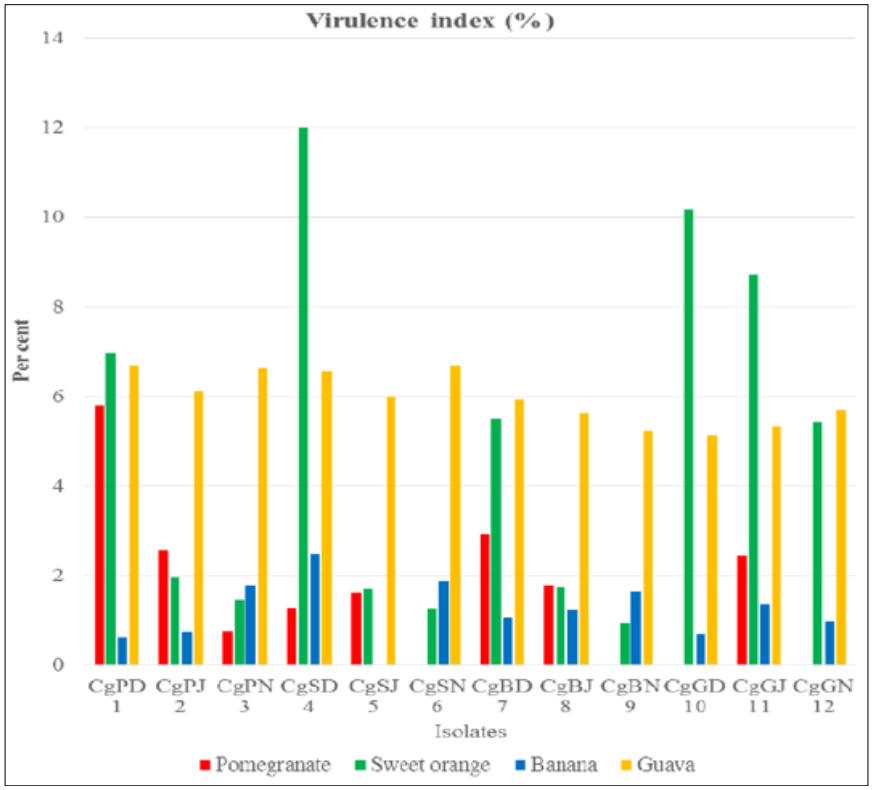

Fig 1: Variation in virulence index of *C. gloeosporioides* isolates and its cross infectivity potential on different fruits

Conclusion

The cross inoculation experiments demonstrated variations in the level of host preference among *C. gloeosporioides* isolates from different fruit crops. Among the different fruit crops, guava and sweet orange were highly susceptible to the anthracnose disease. Among different isolates of *C. gloeosporioides*, the pomegranate, sweet orange isolate was more virulent on guava fruits followed by banana isolates. This study established the possibility of cross infection between host organisms in the case of twelve isolates of *C. gloeosporioides* with respect to examined fruit crops hosts. This could result in the development of model orchards of pomegranate, sweet range, banana and guava mixed cropping systems, with the motto of an integrated, safe and convenient means of reducing post-harvest losses in fruit crops.

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