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Mango seedlings as affected by fungus *Colletotrichum gloeosporioides* through different inoculation methods

Rawal Ahmed Qambrani[1]*, Muhammad Ibrahim Khaskheli[2], Sajad Ali Khaskheli[1] and Zareen Qambrani[3]

[1]Department of Plant Pathology, Sindh Agriculture University Tandojam 70060, Pakistan
[2]Department of Plant Protection, Sindh Agriculture University Tandojam 70060, Pakistan
[3]Department of Biotechnology, Sindh Agriculture University, Tandojam 70060, Pakistan

*Corresponding author: Rawal Ahmed Qambrani (raqambrani@hotmail.com, rawalahmedq@gmail.com)

Abstract

This study aimed to evaluate mango seedlings as affected by fungus *Colletotrichum gloeosporioides* through different inoculation methods. Pathogenicity test was conducted under controlled conditions by using different inoculation methods for confirmation of pathogen, i.e., spraying after rupturing method (M1), spraying method (M2), Injecting method (M3) and Disk method (M4). The data was recorded as plant height, infected number of leaves, numbers and size of lesions after every 15 days of interval 15, 30 and 45 days after inoculation. The maximum disease incidence was recorded in (M1) followed by (M2), (M3) and (M4). Minimum plant height was recorded in (M1) followed by (M2), (M3) and (M4). The maximum number of necrotic spots on plants were recorded in (M1) followed by (M2), (M3) and (M4), whereas no necrotic lesions were recorded in control. Initially, in 15 days minimum necrotic lesions were observed, while maximum necrotic lesions were recorded in 30 and 45 days. The necrotic spots per plant were recorded in (M1) followed by (M2), (M3) and (M4). However, the maximum necrotic spots length for different days was recorded in 45 followed by 30 and 15 days. Whereas, the interaction between methods and days represents the maximum length was recorded 45 days in (M1). The interaction between days after inoculation and methods represents that maximum width of necrotic spots were recorded 45 days in (M1). The total size of necrotic spots (length + width) was recorded in (M1) followed by (M2), (M3) and (M4). The maximum size was recorded in 45 days. The interaction with total (length + width) size of necrotic spots for days and methods represents that the maximum size was recorded 45 days in (M1).

Keywords: Anthracnose, *Colletotrichum gloeosporioides*, Inoculation methods, Mango, Seedlings, Pathogenicity

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Introduction

Mango, (*Mangifera indica* L.) is a significant, delicious, tropical and subtropical fruit in the world, and has a place with family Anacardiaceae. It's positioned first position universally because of significance and that is the reason it's known as the king of the fruits. It is brilliant fruits of tropical and sub-tropical areas and is cultivated in ninety nations of the world. India, China, Mexico, Thailand, Philippines, Pakistan, Nigeria, Indonesia, Brazil, and Egypt are the best ten mango transporting nations. Pakistan is the 6th mango country for the mango production (Gopalakrishnan, 2013).

The mango is one of the most popular fruits and is designated as a national tree (FAO, 2013). In Pakistan, all our territory under mango is 170.3 million hectares with the yield of 175 million tonnes being the second major fruit, produces 8% of the world and basically exports to Middle East, Germany, Japan, Italy and UK, making its valued support as significant foreign currency earning fruit. Pakistani mango is perceived as a standout amongst the best of its sort on the planet (Anonymous, 2016 and Mirza, 2011). Mango is an important outside trade attractive item of Pakistan that makes one of the main mangoes sent out for export in the world (Maqbool et al., 2007).

Mango is affected by several infectious diseases caused by many phytopathogens. Among these all, anthracnose of mango has become one of the serious diseases in recent days and considered serious threats to mango production and quality of fruit. Mango is affected by various diseases at all phases of its development, from seedling in the nursery to the fruit in storage (Ploetz, 2003; Prakash, 2004).

Mango anthracnose is caused by *Colletotrichum gloeosporioides* (Peres et al., 2005; Jayasinghe and Fernando, 2009; Phoulivong et al., 2010). The pathogen represents a danger to mango (Amusa et al., 2005; Masyahit et al., 2009; Owolade, 2009; Erpelding, 2010). The side effects are different oval or irregular symptoms of earthy colored or reflective spots of various sizes scattered wherever all through the leaf surface under wet conditions. The growths become rapidly outlining prolonged dull hued or necrotic parts assessing 20-25 mm in estimation, later rupture and become scourged (Sattar and Malik, 1939).

Materials and Methods

The experiment and studies on mango seedlings as affected by *Colletotrichum gloeosporioides* through different inoculation methods and pathogenicity of mango anthracnose disease causing fungus was conducted in the Mycological Laboratory, Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University Tandojam, Pakistan during 2017 to 2019. The materials and methods carried out as per plan of work are described below.

Pathogenicity test

In order to confirm pathogenicity of the isolated fungus, *Colletotrichum gloeosporiodies*, the pathogenicity test of mango anthracnose disease causing fungus was conducted at seedling stage through different inoculation methods such as: (01) Injecting method; (02) Disk method; (03) Spraying followed by rupturing method; (04) Spraying method. The un-inoculated and untreated mango seedling was kept in control (Fig. 1).

Fig. 1 Pathogenicity test of mango anthracnose disease causing fungus was conducted at seedling stage through different inoculation methods

Preparation of conidial and spore suspension of fungus

The inoculum was used for inoculation of targeted fungus, which made from conidial and spore suspension, prepared from 3 week old pure culture of *Colletotrichum gloeosporioides.* 10 ml sterilized distilled water added in the petri plate of pure culture of fungus *Colletotrichum gloeosporioides* and then rubbed thoroughly with the help of sterilized toothbrush and slides. The contents were transferred in the sterilized beaker and 90 ml of sterilized distilled water were added to make complete 100 ml fungal suspension. The spore and conidial concentration were determined by using a scientific hemocytometer, and the dilution was made to adjust with the load of 2.0 x 10^4 conidia mL^1 concentration with sterilized water.

1. Injecting method

Firstly, the hands were washed with spirit through an atomizer and then pin-sized holes were made with the help of sterilized needle on the stem of mango seedlings. The spore suspension (2.0 x 10^4 conidia mL^1) was inoculated with the help of injection in these holes. Inoculum suspension was injected on the top, middle and lower portion of the seedlings stem. The inoculated seedlings were provided favorable conditions for infection germination and control seedlings were kept without inoculation.

2. Disk method

The seedlings of mango were injured with the help of a sterilized sharp knife at three portions such as top, middle and lower part of the stem. These portions of stem and leaves were inoculated with an agar disc of *Colletotrichum gloeosporioides* pathogen and warped with tape solution. After 2 weeks, the tape solution was removed. The seedlings were observed for development of disease. The 3 seedlings replications were kept as control for compression.

3. Spraying followed by rupturing method

In this method the overall leaves of mango seedlings were ruptured and pinned with the help of sterilized needle, respectively. After then the conidial and spore suspension as described earlier was sprayed over the leaves for the infection of anthracnose disease causing pathogen. Three replications were maintained, and untreated seedlings of mango were used as control.

4. Spraying method

Mango seedlings were inoculated with the spore suspension of *Colletotrichum gloeosporioides* with three replications, respectively. The whole seedling was sprayed with conidia and spore suspension (2.0×10^4 conidia mL^1) for fungus germination. Un-inoculated and untreated three replications of mango seedling were used as control. The experiment was conducted on one year old mango (Desi) seedlings in Complete Randomized Design (CRD) with three replications. The data was observed for mango seedlings height, infected number of leaves, number and size of lesions developed on leaves and stems after every 15 days started through as initial data, after every 15 days, 30 days and 45 days of inoculation for disease development.

Statistical analysis

The data recorded on different parameters was statistically analyzed by using "Statistix 8.1" a statistical package of computer software.

Results

Effect of mango anthracnose disease causing fungus on leaves (disease incidence percent)

The maximum disease incidence (percent of infected leaves) was recorded in spraying after rupturing method (28.095) followed by spraying (19.890), injecting (13.286)) and disk method (6.3425). There are no disease symptoms in control (un-inoculated). Statistically, the results are highly significant and the LSD for all-pairwise comparisons test of incidence percent with different inoculation methods represents five different groups in which the means are highly significant different from one another. The standard error and critical value for comparison is (0.9362 and 1.8953) (Table 1). It is also clear from the results (Table 1), that the incidence percent was increased as the days were increased to the inoculation method. The maximum disease incidence percent was recorded 45 days after inoculation (23.794) followed by 30 and 15 days after inoculation (19.010 and 11.287) there was no disease before inoculation. The LSD for all-pairwise comparisons test also shows that all means are significantly different from one another and the standard error and critical value for comparison is (0.8374 and 1.6952). When the data was analyzed for interaction between methods of inoculation and period (days) for infection, the maximum disease incidence was recorded 45 days after inoculation in case of spraying of inoculum after rupturing (49.427), followed by 30 days after inoculation through spraying after rupturing and 45 days after inoculation through spraying (38.523 and 35.350), followed by 30 days after inoculation through spraying and 15 days after inoculation through spraying after rupturing (27.070 and 24.430) and 45 days after inoculation through injecting (23.187). The minimum disease incidence (4.1800) was recorded 15 days after inoculation through disk method followed by 30 days after inoculation through disk method, 15 days after inoculation through injecting and 45 days after inoculation through disk method (10.183, 10.683 and 11.007) followed by 15 days after inoculation through spraying and 30 days after injecting (17.140 and 19. 273) (Fig. 2). There were no disease symptoms (Fig. 3) before inoculation through any method and in control. However, there are 8 groups in which the means are not significantly different from one another, while standard error and critical value for comparison was 1.8725 and 3.7906.

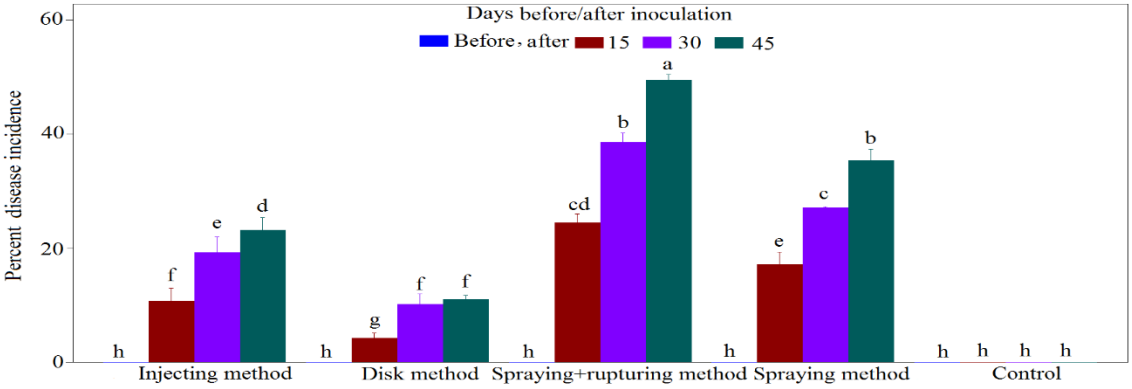

Fig. 2 Effect of mango anthracnose disease causing fungus on leaves (disease incidence percent) interaction between days and methods

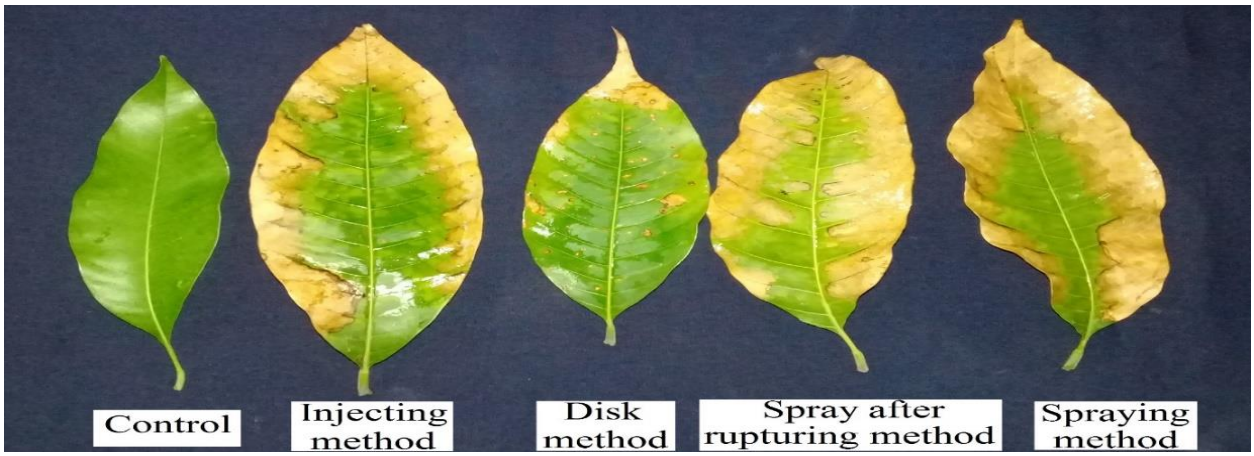

Fig. 3 Effect of mango anthracnose disease causing fungus on leaves

Table 1 Effect of mango anthracnose disease causing fungus on leaves **(**disease incidence percent) mean of methods and days

Methods	Mean	Days	Mean
Spray after rupturing method	28.095 a	45 days after inoculation	23.794 a
Spraying method	19.890 b	30 days after inoculation	19.010 b
Injecting method	13.28 c	15 days after inoculation	11.287 c
Disk method	6.3425 d	Before inoculation	0.0000 d
Control	0.0000 e		

Effect of mango anthracnose disease causing fungus on plant height (cm)

The anthracnose disease causing fungus significantly reduced plant height. The minimum plant height was recorded in the spraying after rupturing and spraying method (62.917 and 64.000) followed by injecting (65.5.83), and disk method (67.000). Whereas, the maximum plant height was recorded in the un-inoculated plants (control, 68.333) as compared to inoculation through different methods (Table 2). There are four groups in which the means are not significantly different from one another and the standard error and critical value for comparison is (0.5820 and 1.1782). The Comparisons of plant height for different days also represents that as the days were increased the plant height was increased but all 4 means are highly significant different from one another. The maximum mean plant height (68.600) with different methods of inoculation was recorded 45 days after inoculation, followed by 30 days (67.133) and 15 days (64.667) after inoculation (and minimum mean plant height (61.867) was recorded at the time of inoculation/before inoculation (Table 2). The standard error and critical value for comparison is (0.5206 and 1.0538). The interaction between method of inoculation and period (days before and after inoculation) represents 10 groups in which the means are not significantly different from one another at alpha 0.05 where standard error and critical value for Comparison was 1.1640 and 2.3565. Significantly maximum mean plant height was recorded in un inoculated plants 45 days after inoculation day (75.333) followed by inoculation through disk method 45 days after inoculation (71.000), Control 30 days after inoculation, Disk 30 days after inoculation, Injecting 45 days after inoculation, Injecting 30 days after inoculation and Control 15 days after inoculation and Disk 15 days after inoculation (70.667, 69.333, 68.333, 67.333, 66.000 and 65.667), respectively. While 64.667 plant height was recorded in case of Injecting 15 days after inoculation and spraying 30 and 45 days after inoculation followed by 64.333 in Spraying 15 days after inoculation and 63.667 in Spraying after Rupturing 30 and 45 days after inoculation. The minimum (61.333 and 61.667) mean plant height was recorded before/at the time of inoculation in control and spraying after rupturing followed by disk and injecting method (62.000), spraying (62.333) before/at the time of inoculation and spraying after rupturing (62.667) at 15 days after inoculation (Fig. 4).

Table 2 Effect of mango anthracnose disease causing fungus on plant height (cm) mean of methods and days

Methods	Mean	Days	Mean
Control	68.333 a	45 days after inoculation	68.600 a
Disk method	67.000 b	30 days after inoculation	67.133 b
Injecting method	65.583 c	15 days after inoculation	64.667 c
Spraying method	64.000 d	Before inoculation	61.867 d
Spray after rupturing method	62.917 d		

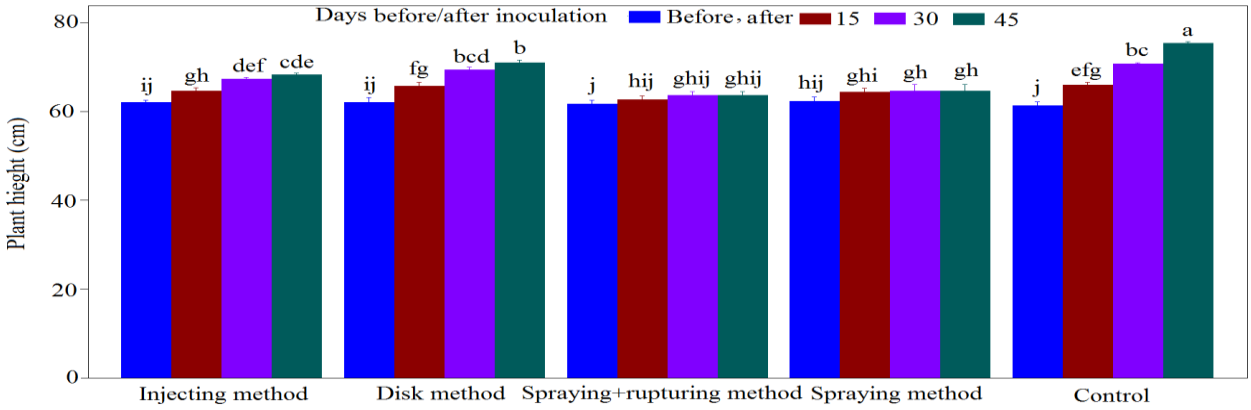

Fig. 4 Effect of mango anthracnose disease causing fungus on plant height (cm) interaction between days and methods

Effect of mango anthracnose disease causing fungus on necrotic spots per plant

The maximum number of necrotic spots on plants were recorded in spraying after rupturing method (15.333) followed by spraying (10.222), injecting method (6.8889) and disk method (4.6667), whereas no necrotic lesions were recorded in control (Table 3). LSD 0.05 for all-pairwise comparisons test represents that all 5 means are significantly different from one another. The standard error and critical value for comparison is (0.8727 and 1.7876). It was also observed that as the days after inoculation increased, the number of necrotic lesions also increased. Initially, i.e. 15 days after inoculation, 5.4667 necrotic lesions were recorded, while 7.5333 and 9.2667 necrotic lesions were recorded 30 and 45 days after inoculation (Table 3). All the 3 means are significantly different from one another and the standard error and critical value for comparison is 0.6760 and 1.3847. The interaction between method of inoculation and days after inoculation represents 8 groups in which the means are not significantly different from one another, standard error and critical value for comparison was 1.5115 and 3.0962. The maximum number of necrotic spots (19.667) were observed 45 days after inoculation through spraying after rupturing followed by spraying after rupturing, spraying, spraying after rupturing and spraying (15.667, 13.000, 10.667 and 10.000), 30, 45, 15 and 30 days after inoculation, respectively (Fig. 5). There were no necrotic spots in control at 15, 30 and 45 days after inoculation (Fig. 6). Whereas, minimum number of necrotic spots (3.000) were observed 15 days followed by 5.000 spots 30 days after inoculation through disk method, 5.667 spots in case of injection and disk methods 30 and 15 days after inoculation, 8.0000, 7.6667 and 7.0000 spots from Injecting, Spraying and Injecting method 45, 15 and 30 days after inoculation, respectively (Fig. 4).

Table 3 Effect of mango anthracnose disease causing fungus on necrotic spots per plant (mean of methods and days)

Methods	Mean	Days	Mean
Spray after rupturing method	15.333 a	45 days after inoculation	9.2667 a
Spraying method	10.222 b	30 days after inoculation	7.5333 b
Injecting method	6.8889 c	15 days after inoculation	5.4667 c
Disk method	4.6667 d		
Control	0.0000 e		

Effect of mango anthracnose disease causing fungus on length (cm) of necrotic spots per leaf

The maximum mean length (4.2222) of the necrotic spots per plant was recorded in spraying after rupturing method followed by (3.5111) in spraying, (2.7767) injecting and 1.9044 spots in disk method but there was no necrotic lesion length (0.0000) in control (Table 4). The LSD 0.05 for all-pairwise comparisons test results that all 5 means are significantly different from one another where the standard error and critical value for comparison was 0.1474 and 0.3019. However, the maximum mean necrotic spots length for different days (3.7273) was recorded in 45 days after inoculation followed by 2.4640 and 1.2573 was recorded in 30 and 15 days after inoculation (Table 4). All three means are highly significantly different from one another, standard error and critical value for comparison is 0.1142 and 0.2339. Whereas, the interaction between method of inoculation and period in days after inoculation represents that there are 9 groups in which the means are not significantly different from one another. The maximum mean length was recorded 45 days after inoculation through Spray after Rupturing (5.9733) followed by 45 days after inoculation through spraying (5.0300), 30 days after inoculation through Spray after rupturing and 45 days after inoculation through Injecting method (4.4533 and 4.4033), 30 days after inoculation through Spraying and 45 days after inoculation through Disk method (3.5600 and 3.2300). The minimum mean length of necrotic spots (0.7367) were recorded in 15 days after

inoculation with Disk method followed by 1.3667 in 15 days after inoculation through Injecting, 1.7467 in30 days after inoculation Disk method, 1.9433 in15 days after inoculation through Spraying method, 2.2400 in 15 days after inoculation with Spray after Rupturing and 2.5600 in 30 days after inoculation through Injecting method. Whereas, there were no necrotic spots in control (Fig. 7). The standard error and critical value for comparison was 0.2553 and 0.5229.

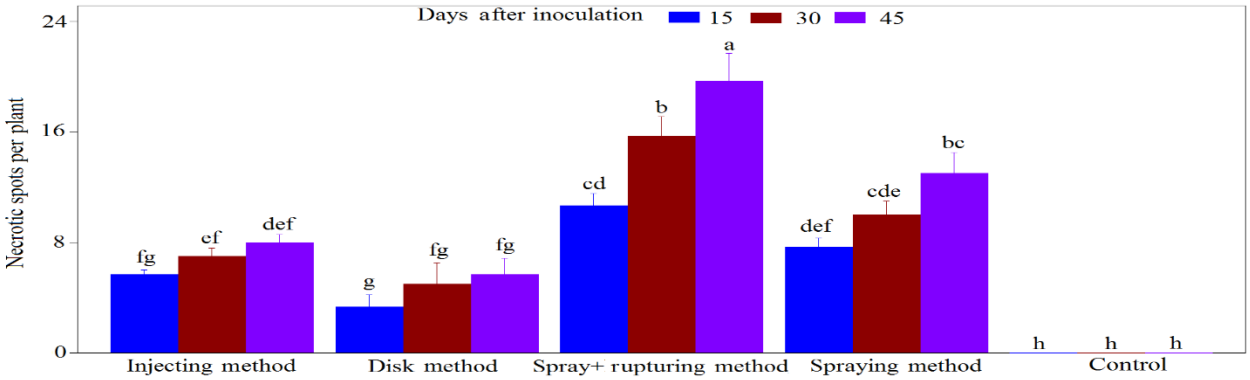

Fig. 5 Effect of mango anthracnose disease causing fungus on necrotic spots per plant (interaction between days and methods)

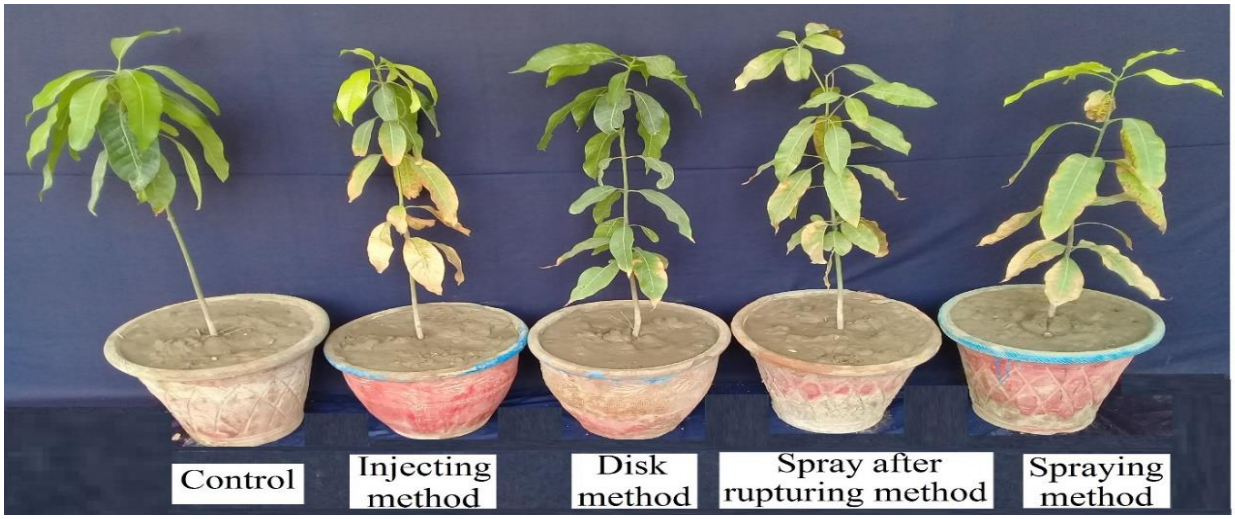

Fig. 6 Effect of mango anthracnose disease causing fungus on necrotic spots per plant

Table 4 Effect of mango anthracnose disease causing fungus on length (cm) of necrotic spots per leaf (mean of methods and days)

Methods	Mean	Days	Mean
Spray after rupturing method	4.2222 a	45 days after inoculation	3.7273 a
Spraying method	3.5111 b	30 days after inoculation	2.4640 b
Injecting method	2.7767 c	15 days after inoculation	1.2573 c
Disk method	1.9044 d		
Control	0.0000 e		

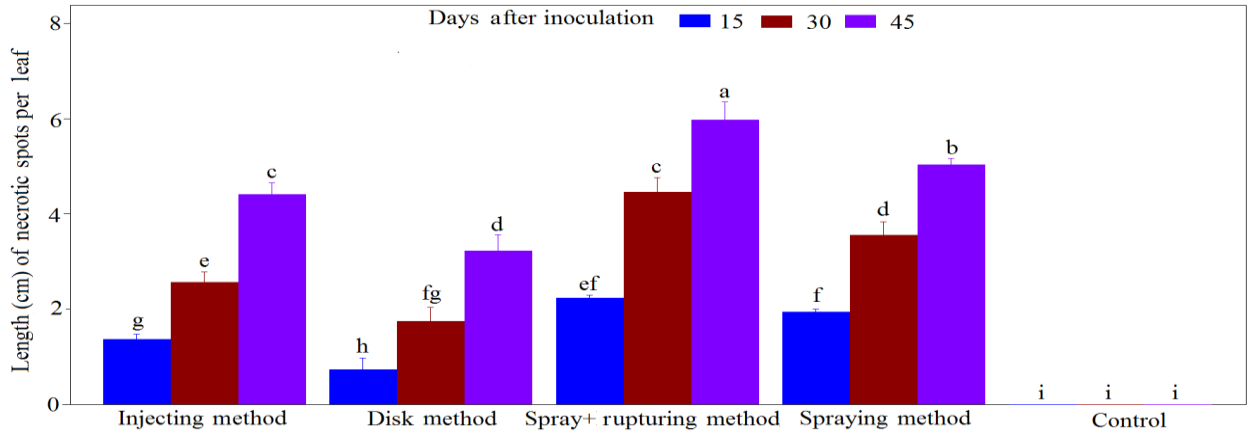

Fig. 7 Effect of mango anthracnose disease causing fungus on length (cm) of necrotic spots per leaf (interaction between days and methods)

Effect of mango anthracnose disease causing fungus on width (cm) of necrotic spots per leaf

The maximum mean width of necrotic spots (4.4633) was recorded in spraying after rupturing followed by spraying (3.7433), injecting (2.9700) and disk method (2.3289). There were no necrotic spot (0.0000) in control because there was no inoculation (Table 5). All five means are significantly different from one another. The standard error and critical value for comparison is (0.1945 and 0.3984). With reference to days after inoculation, the maximum mean width of necrotic spots (3.4733) was recorded at 45 days after inoculation followed by 30 days (2.7253) and 15 days after inoculation (1.9047) (Table 5). All 3 means are significantly different from one another. The standard error and critical value for comparison is (0.1507 and 0.3086). The interaction between days after inoculation and method of inoculation represents that the maximum mean width of necrotic spots (5.5267) were recorded 45 days after inoculation through spraying after rupturing method followed by 45 days after inoculation through spraying method (4.7500), 30 days after inoculation through spraying after rupturing method (4.4433), 45 days after inoculation through injecting and 30 days after inoculation through spraying method (3.8600 and 3.7900), followed by 15 days after inoculation through spraying after rupturing method (3.4200), 45 days after inoculation through disk method (3.2300), whereas minimum mean width of necrotic spots (1.4000) 15 days after inoculation through disk method followed by 15 days after inoculation through injecting method (2.0133), 30 days after inoculation through disk method (2.3567), 15 days after inoculation through spraying method (2.6900) and 30 days after inoculation through injecting method (3.0367), but there were no necrotic spots in control (Fig. 7). However, there are 10 groups in which the means are not significantly different from one another, standard error and critical value for comparison was 0.3369 and 0.6901.

Table 5 Effect of mango anthracnose disease causing fungus on width (cm) of necrotic spots per leaf (mean of methods and days)

Methods	Mean	Days	Mean
Spray after rupturing method	4.4633 a	45 days after inoculation	3.4733 a
Spraying method	3.7433 b	30 days after inoculation	2.7253 b
Injecting method	2.9700 c	15 days after inoculation	1.9047 c
Disk method	2.3289 d		
Control	0.0000 e		

Effect of mango anthracnose disease causing fungus on size length+width (cm) necrotic spots per leaf

The total size of necrotic spots (length + width) for different inoculation methods represents that all the 5 means are highly significant from one another. The maximum mean size (8.6856) was recorded in spraying after rupturing method of inoculation, followed by spraying method (7.2544), injecting (5.7467) and disk method of inoculation (4.2333). There were no necrotic spots 0.0000) in control (Table 6). The standard error and critical value for comparison is 0.2127 and 0.4358. In the case of the time period (Table 6), the maximum mean size (7.2007) was recorded in 45 days after inoculation followed by 30 days (5.1893) and 15 days after inoculation (3.1620). These all 3 means are significantly different from one another. The standard error and critical value for comparison is 0.2127 and 0.4358. The interaction with reference to the total (length + width) mean size of necrotic spots for days taken after different inoculation methods represents that all the 11 groups in which the means are not significantly different from one another. The maximum mean size (11.503) was recorded 45 days after inoculation through Spraying after rupturing followed by 45 days after inoculation through Spraying (9.7833), 30 days after inoculation through Spraying after rupturing (8.8933), 45 days after inoculation Injecting (8.2600), 30 days after inoculation

Spraying (7.3467), 45 days after inoculation Disk (6.4567), 15 days after inoculation Spraying after Rupturing (5.6600), 30 days after inoculation Injecting (5.6000), 15 days after inoculation Spraying (4.6333), 30 days after inoculation Disk (4.1067), 15 days after inoculation Injecting (3.3800) and 15 days after inoculation Disk (2.1367), whereas, there were no necrotic spots (length + width) in control (Fig. 8). The standard error and critical value for comparison was 0.4757 and 0.9744.

Table 6 Effect of mango anthracnose disease causing fungus on size length+width (cm) necrotic spots per leaf (mean of methods and days)

Methods	Mean	Days	Mean
Spray after rupturing method	8.6856 a	45 days after inoculation	7.2007 a
Spraying method	7.2544 b	30 days after inoculation	5.1893 b
Injecting method	5.7467 c	15 days after inoculation	3.1620 c
Disk method	4.2333 d		
Control	0.0000 e		

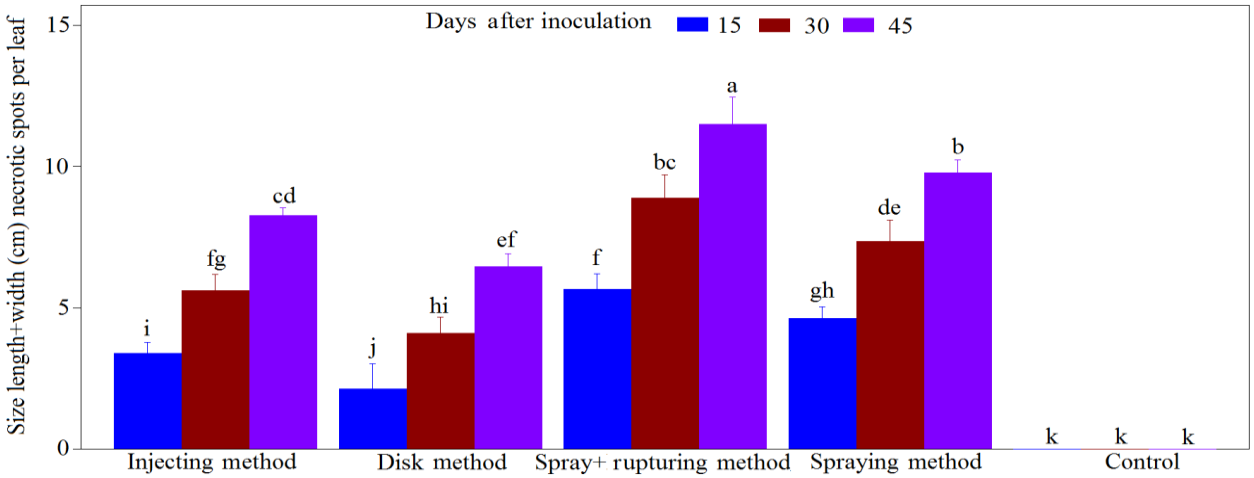

Fig. 8 Effect of mango anthracnose disease causing fungus on total size length + width (cm) necrotic spots per leaf **(**interaction between days and methods)

Discussion

The results with reference to mango seedlings as affected by *Colletotrichum gloeosporioides* through different inoculation methods represents a highly significant difference. The maximum disease incidence (percent of infected leaves**),** reduced plant height, maximum number of necrotic spots, length, width and total necrotic size was recorded in spraying after rupturing method followed by spraying, injecting and disk method. The incidence was increased as the days were increased to the inoculation method. The plant height was decreased as the days were increased to the inoculation method. The number of necrotic spots, length, width and total necrotic size was increased as the days were increased to the inoculation method. The interaction between methods of inoculation and period (days) for infection resulted that maximum disease incidence, reduced plant height, maximum number of necrotic spots, length, width and total necrotic size was recorded 45 days after inoculation in case of spraying of inoculum after rupturing.

Mo et al. (2018), Rakesh and Singh (2017), Abera et al. (2016), Lurwanu et al. 2014; Naqvi al. (2014), Bhuvaneswari and Rao (2001), Davis (1999), Freeman and Katan (1997), Kumar (1997), Agostini et al. (1992), Chandra and Pathak (1992), Dodd et al. (1991), Fitzell et al. (1984), Spalding (1982), Fitzell (1979) also conducted several studies on the isolation of causal pathogens from different mango parts and were confirmed through pathogenicity tests and reported near about similar results with reference to reported and other parameters.

Conclusion

It is concluded from present investigations on mango seedlings as affected by Fungus *Colletotrichum gloeosporioides* through different inoculation methods. The maximum disease incidence (28.095) was recorded in spraying after rupturing method 45 days after inoculation significantly reduced plant height (62.917 and 64.000) maximum number of necrotic spots on plants followed by spraying method (7.2544), injecting (5.7467) and disk method of inoculation (4.2333). In the case of the time period, the maximum mean size (7.2007) was recorded in 45 days after inoculation.

Conflict of Interest: The authors declare that they have no conflict of interest.

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