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## TEMPORAL PROPHYLAXIS EVALUATION AND GERMPLASM SCREENING AGAINST *COLLETOTRICHUM CIRCINANS* CAUSING ONION SMUDGE

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### ABSTRACT

Smudge is a common disease of onion that can appear in the fields as well as during crop storage or transportation after harvesting. It is mainly a bulb disease that can affect the crop's appearance and market value. Among different treatments evaluated as pre- and post-inoculation spray, mean maximum incubation period (212.00 h) was recorded in *Datura* sp. which was significantly followed by *Ocimum* sp. (160.80 h) irrespective of type of sprays. However, mean minimum incubation period was recorded in azoxystrobin 18.2% + Difenconazole 11.4% (36.00 h) which was statistically at par with mancozeb 75% (44.00 h). Onion varieties with yellow and red coloured outer scales exhibited resistance towards onion smudge, and at the stage before the development of pigment in the outer scales, the seedlings of coloured varieties are also susceptible to the disease. Protocatechuic and catechol present in pigmented varieties were toxic to the causal fungus and impacted resistance to the disease. Among different varieties screened for disease resistance, mean maximum lesion diameter (51.25 mm) was recorded in N-53 and the minimum (6.50 mm) was recorded in PWO-2. As far as incubation period was concerned, minimum incubation period (30.00 h) was recorded in N-53 and maximum (132.00 h) was in XP-Red. Only white coloured and light pink coloured onions were infected by the pathogen as red coloured onion cultivars were resistant to the disease.

**Keywords:** Smudge, inoculation, varieties, resistance, incubation period, screened, protocatechuic acid and catechol.

### Introduction

Onion production and marketing are affected by more than a dozen diseases caused by fungi, bacteria and viruses (Kiehr *et al.*, 1996; Kiehr and Delhey, 2007), with onion smudge being an important disease of white and yellow onions. This fungus was first described in the United Kingdom and has since spread around the world, especially in the Northern Hemisphere. Several *Colletotrichum* spp. have been reported as disease causing agents of onion smudge

(Farr and Rossman, 2012). In highly warm and wet conditions only, the young seedlings are attacked however, actively growing portions of seedlings are not attacked. In field conditions, *C. circinans* survives on the scales and leaves. After harvesting, dormant cells start to enter the fleshy scales of the bulbs. The disease normally spreads slowly, but in a warm and moist environment, it may occur more rapidly leading to the decay of bulb (Scheck, 2021). The most effective and corrective measures for the white onion set crop is early harvesting and using all practices to protect the

crop from rainy weather. Storage in ventilated warehouse at freezing reduces the disease incidence (Walker, 1921). Hexaconazole and mancozeb were found to be very effective in reducing the diameter of the lesion and spore germination (Patel *et al.*, 2005).

Despite the fact that *Colletotrichum* spp. can be controlled by using commercially available fungicides (Barbate *et al.*, 2012), only limited success has been attained because pathogen-specific chemicals are not readily available. This has resulted in the increased level of tolerance in the fungus, the emergence of resistant strains and the appearance of new toxicant-tolerant pathogen races. Management approaches that are secure, environmental friendly and economically feasible must be explained in order to control crop plant diseases. Chemicals are known to have adverse effect when used continuously and inappropriately. Such outcomes result in persistent toxicity, resistance, environmental contamination and risks to human and animal health. Alternative forms of control have been implemented in an effort to change this circumstance. Currently, the use of plant extracts with toxic effects against the phytopathogen is being investigated. Because of their simple breakdown, lack of environmental pollution, lack of residual toxicity and lack of phytotoxic properties, they are being evaluated for controlling the phytopathogens (Dixit *et al.*, 1979).

The most affordable, simple, secure and efficient method of disease management is the use of resistant cultivars. This is done in order to reduce pollutants carried on by the use of harmful chemicals, eliminate disease-related losses and lower the expense of chemical and mechanical control (Park, 2007). Additionally, research has been carried on potential biological control utilising biopriming techniques.

According to Walker (1923), onion varieties with yellow and red coloured outer scales exhibited resistance towards onion smudge and at the stage before the development of pigment in the outer scales, the seedlings of coloured varieties are also susceptible to the disease. Later, Link *et al.* (1929), Angell *et al.* (1930), Walker *et al.* (1929) and Link and Walker (1933) reported that protocatechuic and catechol present in pigmented varieties were toxic to be the causal fungus and impacted resistance to the disease.

### Material and Methods

The study was conducted during 2020-2022 under laboratory conditions in the Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India

### Isolation, identification and pathogenicity of the Pathogen

The diseased samples of onion showing typical symptoms of smudge were repeatedly washed in tap water. Thereafter, small pieces of the diseased tissues from infected portion of bulb were taken, along with some healthy tissue with the help of sterilized blade and surface sterilized with 1 per cent sodium hypochlorite. The tissue were placed on the potato dextrose agar (PDA) medium poured in sterilized Petri plates and slants with sterilized inoculating needle. The inoculated plates as well as slants were incubated at  $28\pm 2^{\circ}\text{C}$  in BOD incubator. After the initiation of the fungal growth in the inoculated plates/ slants, an agar bit from the periphery of actively growing mycelium was taken and placed on sterilized PDA slants to purify the culture. These purified cultures were then maintained in the refrigerator at  $4-5^{\circ}\text{C}$  for use in further experiments. The pathogen was identified on the basis of cultural and microscopic characters.

### Effect of pre and post inoculation spray treatments on the development of disease.

Mature and healthy onion bulbs procured from market were first washed under tap water thoroughly. They were then wiped with 1 per cent sodium hypochlorite solution and again rinsed with sterilized distilled water. These bulbs were then sprayed with different chemicals/botanicals viz., myclobutanil 10%, tricyclazole 75%, metiram 55% + pyraclostrobin 5%, azoxystrobin 18.2% + difenconazole 11.4%, carbendazim 50%, mancozeb 75% and metiram + zinc 14% were evaluated at their field concentrations and three effective botanicals viz., *Ocimum* sp., *Datura* sp. and *Murraya* sp., were evaluated at 20 per cent as pre and post inoculation spray before and after 2 hours of inoculation with culture of pathogen. These bulbs were then incubated at room temperature to see the effect of different treatments on the development of onion smudge. Bulbs sprayed with sterilized distilled water and inoculated with pathogen culture served as check. Data were recorded in terms of incubation period (h) and lesion diameter (mm).

### Screening of germplasm

Five onions of different available commercial varieties/hybrids viz., PWO-2, N-53, Palam Lohit, RANI F1, XP-Red were screened under artificial inoculation conditions for susceptibility or resistance towards disease. For these, ten bulbs of each variety were washed under tap water thoroughly. These were then wiped with 1 per cent sodium hypochlorite solution and again rinsed with sterilized distilled water twice. These fruits were then inoculated with test

pathogen and placed in dessicators at room temperature so as to record the incubation period and lesion diameter. The varieties were categorized as highly resistant to highly susceptible as per the following scale in Table 1.

### Statistical Analysis

Laboratory experiments were conducted with three to four replications in each treatment and results were statistically analysed by using Completely Randomized Design (CRD) as per Panse and Sukhatme (2000) by using online programme OPSTAT (Sheoran *et al.*, 1998).

## Results and Discussion

### Collection, isolation and identification of the pathogen

The pathogen was isolated from a symptomatic onion bulb (Fig. 1a). The symptoms on infected bulb were recorded as small black spots on outer scales in circinate manner or concentric rings. The fruiting bodies were formed on the stromata which developed beneath the cuticle of the host. One to several acervuli were formed on a single stroma. Microscopically, short and hyaline conidiophores were formed in a palisade layer and ruptured the cuticle of the host. The pathogen was isolated and purified on potato dextrose agar (PDA) medium and maintained in PDA slants at 28-30 °C in the refrigerator. The cultural and microscopic characteristics of the pathogen were studied in detail. The mycelium was creamish white in colour having dense, cottony and suppressed growth on PDA (Fig. 1b). The conidiophores were intermixed with brown coloured setae in between (Fig. 1c). Microscopically, the mycelium was hyaline, septate and branched (Fig. 1d). The conidia were slightly curved or falcate and measured between 11.7-15.6 µm in length and 3.9-7.8 µm in width (Fig. 1e). However, in culture no acervuli were produced. Based on microscopic and cultural characters, the associated pathogen was identified as *Colletotrichum circinans* (Berk.) Voglino. The results are in conformity with the findings of Walker 1921, Kiehr *et al.*, 2012 and Leylaie *et al.*, 2014.

### Effect of Pre and Post Inoculation Spray Treatments on the Development of Disease

Seven effective chemicals viz., myclobutanil 10%, tricyclazole 75%, metiram 55% + pyraclostrobin 5%, azoxystrobin 18.2% + difenoconazole 11.4%, carbendazim 50%, mancozeb 75% and metiram + zinc 14% were evaluated at their field concentrations and three effective botanicals viz., *Ocimum* sp., *Datura* sp. and *Murraya* sp., were evaluated at 20 per cent concentration for pre and post inoculation spray

treatments. In case of pre inoculation spray, onion bulbs were first sprayed with treatments and after 2 hours, these bulbs were inoculated with culture of *C. circinans*. While, in case of post inoculation spray, onion bulbs were first inoculated with culture of *C. circinans* and after 2 hours, onions were sprayed with treatments to see the effect of spray treatments on the development of disease. Observations were recorded in terms of incubation period (h) and lesion diameter (mm) after 5 days of symptom appearance and data recorded have been presented in Table 4.2.

A perusal of data presented in Table 4.2. indicate that the mean maximum incubation period (212.00 h) was recorded in *Datura* sp. which was significantly followed by *Ocimum* sp. (160.80 h) irrespective of time of sprays. However, mean minimum incubation period was recorded in azoxystrobin 18.2% + difenoconazole 11.4% (36.00 hours) which was statistically at par with mancozeb 75% (44.00 hours). Irrespective of treatments, incubation period in pre inoculation (104.21 hours) spray was significantly longer than in post inoculation spray (89.16 hours). Body of the table also reveals that significantly longest incubation period (232.00 h) was recorded in bulbs sprayed with *Datura* sp. extract prior to inoculation followed by same spray (192.00 h) given after the inoculation. Shortest incubation period (32.00 h) next to control (24.00 h) was however, recorded in bulbs sprayed with azoxystrobin 18.2% + difenoconazole 11.4% when sprayed after inoculation which was statistically at par with mancozeb 75% (40.00 h) sprayed after inoculation (Figure 4). An intermediate range of incubation period was recorded in rest of the treatments (Figure 2 and Figure 3). As far as lesion diameter was concerned, irrespective of the time of spray, smallest mean lesion diameter (12.47 mm) was recorded in bulbs treated with *Datura* sp. extract followed significantly by those treated with *Ocimum* sp. extract (13.91 mm). However, significantly largest (46.63 mm) mean lesion diameter next to control (49.00 mm) was recorded in bulbs sprayed with azoxystrobin 18.2% + difenoconazole 11.4% followed by those sprayed with mancozeb 75% (38.09 mm). Irrespective of spray treatment, significantly smallest lesion diameter (28.62 mm) was recorded with pre-inoculation spray in comparison to post inoculation spray (30.61 mm) (Figure 5). Body of table revealed that significantly smallest lesion diameter (10.97 mm) was recorded in bulbs sprayed with the *Datura* sp. extract prior to inoculation followed by those sprayed with *Ocimum* sp. extract (12.60 mm) before inoculation which was statistically at par with lesion diameter recorded in bulbs treated with *Datura* sp. extract after inoculation (13.97 mm). Largest lesion

diameter (47.50 mm) was recorded in bulbs treated with azoxystrobin 18.2% + difenoconazole 11.4% sprayed after inoculation. An intermediate lesion diameter was recorded in rest all the treatments under study.

During present studies, pre and post inoculation sprays with *Datura* sp., *Ocimum* sp. and *Murraya* sp. were found to prolong the incubation period and reduce the lesion diameter to a maximum extent under artificial inoculation conditions. Antifungal properties of *Ocimum* spp. have earlier been reported against *C. musae*, the casual agent of banana anthracnose Madjouko *et al.* (2019), which is supportive for our findings. Further, the antifungal properties of *M. koenigii* and *Datura* spp. against different plant pathogens have also been reported by Afzal *et al.* (2014) and ARIK U O (2017) which also support our findings. Among the pre and post inoculation spray treatments, pre inoculation sprays proved better than post inoculation spray by prolonging the incubation period and reducing the lesion diameter. It is a well known fact that *Colletotrichum* spp. infect the crop plants prior to harvest and express their symptoms on produce, after the harvest. So, pre harvest sprays prove effective in managing the disease after harvest. During present studies too, pre inoculation sprays might have inhibited the growth of the fungus and thus prolonged the incubation period in comparison to untreated control in all the treatments.

### Screening of Germplasm

Ten bulbs of different available varieties/hybrids viz., PW0-2, N-53, Palam Lohit, RANI F1 and XP-Red procured from market were screened under artificial inoculation conditions for susceptibility or resistance towards disease. For screening of varieties, test pathogen was inoculated on onion bulbs and incubated at 30°C at 100 per cent RH in desiccators. The data recorded in terms of incubation period (h) and lesion diameter (mm) have been presented on Table 3.

It is evident from the table that shortest incubation period (30.00 h) was recorded in N-53 which was statistically at par with that recorded in Palam Lohit (36.00 h) and PWO-2 (39.00 h). However, significantly longest incubation period (132.00 h) was recorded in XP-Red followed by incubation period in RANI F1 (66.00 h). As far as lesion diameter (mm) was concerned, significantly minimum lesion diameter (6.50 mm) was recorded in XP-Red followed by RANI F1 (25.00 mm) and PW0-2 (37.25 mm) (Figure 6). However, maximum lesion diameter was recorded in N-53 (51.25 mm) followed by lesion diameter recorded in Palam Lohit (46.25 mm). Among all the five

varieties screened, only one i.e., XP-red was categorized as resistant variety based on the lesion diameter while, RANI F1 was categorized as susceptible. Rest three were categorized as highly susceptible varieties (Figure 7) and (Figure 8). The present findings were in accordance with Walker (1923) who concluded that pigmented varieties are resistant towards disease while, non-pigmented varieties are susceptible towards disease. The results are further supported by Link *et al.* (1989), Angell *et al.* (1930), Walker *et al.* (1929) and Link and Walker (1933) who reported that protocatechuic and catechol present in pigmented varieties were toxic to the causal fungus and imparted resistance to the disease.

### Conclusion

From these results, it was concluded that onion smudge is an important disease which occurs in mild to severe form in non-pigmented onion bulbs while, pigmented bulbs are resistant to disease. Symptoms appeared to be small round dark spots scattered over the surface of bulb in concentric rings of diameter 1-2 cm. The fungus grew well on potato dextrose agar having pH 7.0 at 30°C. Temperature 30°C and relative humidity 100 per cent was most favourable for disease development. In-vitro evaluation of plant extracts against pathogen revealed maximum inhibition with *Datura* sp. The pathogen was inhibited effectively in vitro by the use of myclobutanil 10%, tricyclazole 75% and metiram 55% + pyraclostrobin 5% at all concentrations tested and metiram + zinc 14% at 2000 ppm. Among different types of sprays, pre inoculation spray was better as compared to post inoculation spray. Pigmented cultivars exhibited resistance towards the disease while, non-pigmented cultivars were susceptible the disease.

### Further Scope

The present study provides valuable insights into the role of pre and post harvest sprays in managing the disease after harvest and the role of biochemicals present in the red coloured cultivars of onions which are toxic to the pathogen. However, further research is warranted to elucidate the underlying physiological and molecular mechanisms. Detailed studies on survival mechanisms (soil, seed, crop residues, storage conditions), understanding the role of climate change (temperature, humidity, rainfall) on disease incidence, development of forecasting and prediction models to identify critical infection periods and Integrated Disease Management tools may offer new avenues for studying the disease and managing it properly to avoid economic losses.

**Table 1 :** Disease severity scale

Disease level	Lesion diameter (mm)
Highly resistant	0-5
Resistant	5-10
Moderately resistant	10-15
Moderately susceptible	15-20
Susceptible	20-25
Highly susceptible	> 25

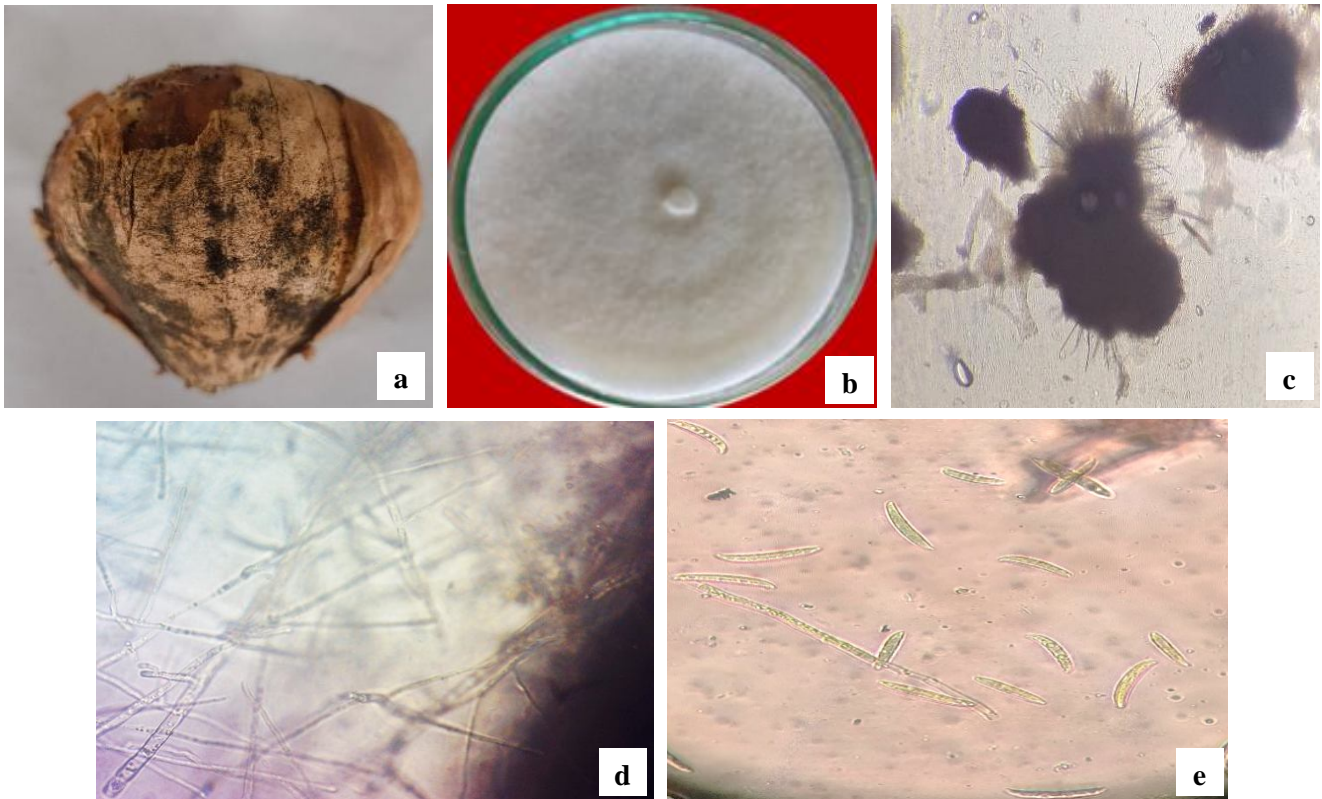
**Table 2:** Effect of pre and post inoculation spray treatments on the development of disease

Chemical / Plant extract	Concentration (%)	Incubation period in spray (hours)			Lesion diameter (mm)		
		Pre inoculation spray	Post inoculation spray	Overall mean	Pre inoculation spray	Post inoculation spray	Overall mean
*Myclobutanil 10%	0.04	128.00	120.00	124.00	25.43	27.33	26.39
*Tricyclazole 75%	0.10	112.00	96.00	104.00	26.00	27.07	26.53
Metiram 55% + Pyraclostrobin 5%	0.30	72.00	60.00	66.00	25.70	26.27	25.99
*Azoxystrobin 18.2% + Difenconazole 11.4%	0.05	40.00	32.00	36.00	45.77	47.50	46.63
Mancozeb 75%	0.25	48.00	40.00	44.00	37.13	39.03	38.09
Metiram + Zinc 14%	0.20	90.40	72.00	81.20	31.63	34.30	32.97
*Carbendazim 50%	0.05	112.00	83.20	97.60	29.70	32.10	30.90
<i>Datura</i> sp.	20	232.00	192.00	212.00	10.97	13.97	12.47
<i>Ocimum</i> sp.	20	168.00	153.60	160.80	12.60	15.23	13.91
<i>Murraya</i> sp.	20	120.00	108.00	114.00	20.97	24.93	22.95
Control	-	24.00	24.00	24.00	49.00	49.00	49.00
<b>Overall mean</b>		104.21	89.16			30.61	
		<b>Factors</b>	<b>CD<sub>P≥0.05</sub></b>	<b>SE<sub>(d)</sub></b>	<b>Factors</b>	<b>CD<sub>P≥0.05</sub></b>	<b>SE<sub>(d)</sub></b>
		Treatment	10.63	5.26	Treatment	1.00	0.49
		Concentration	4.53	2.24	Concentration	0.42	0.21
		Interaction	15.04	7.43	Interaction	1.42	0.70

\*Concentrations tested were 125, 250, 375 and 500 ppm

**Table 3 :** Screening of varieties for susceptibility or resistance towards disease

Varieties	Incubation period (days)	Lesion diameter (mm)	Category
PWO-2	1.62	37.25	Highly susceptible
N-53	1.25	51.25	Highly susceptible
Palam Lohit	1.50	46.25	Highly susceptible
RANI F1	2.75	25.00	Susceptible
XP-Red	5.50	6.50	Resistant
<b>CD<sub>P≥0.05</sub></b>	0.80	2.52	
<b>SE<sub>(d)</sub></b>	0.37	1.17	



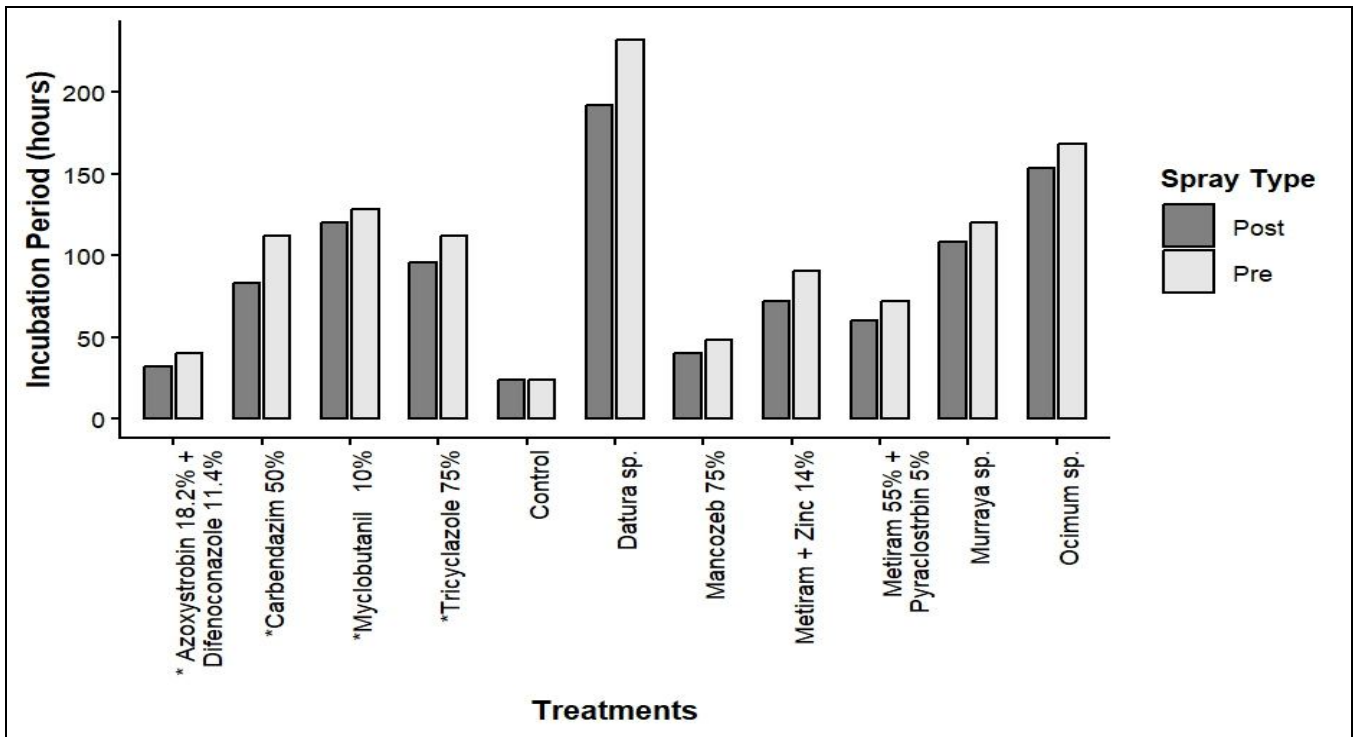
**Fig. 1:** (a) An onion bulb exhibiting symptoms of smudge (b) Pure culture of *Colletotrichum circinans* from host tissue (c) Setae of *Colletotrichum circinans* (d) Mycelium of *Colletotrichum circinans* from host tissue (e) A micrograph of *Colletotrichum circinans* exhibiting conidia



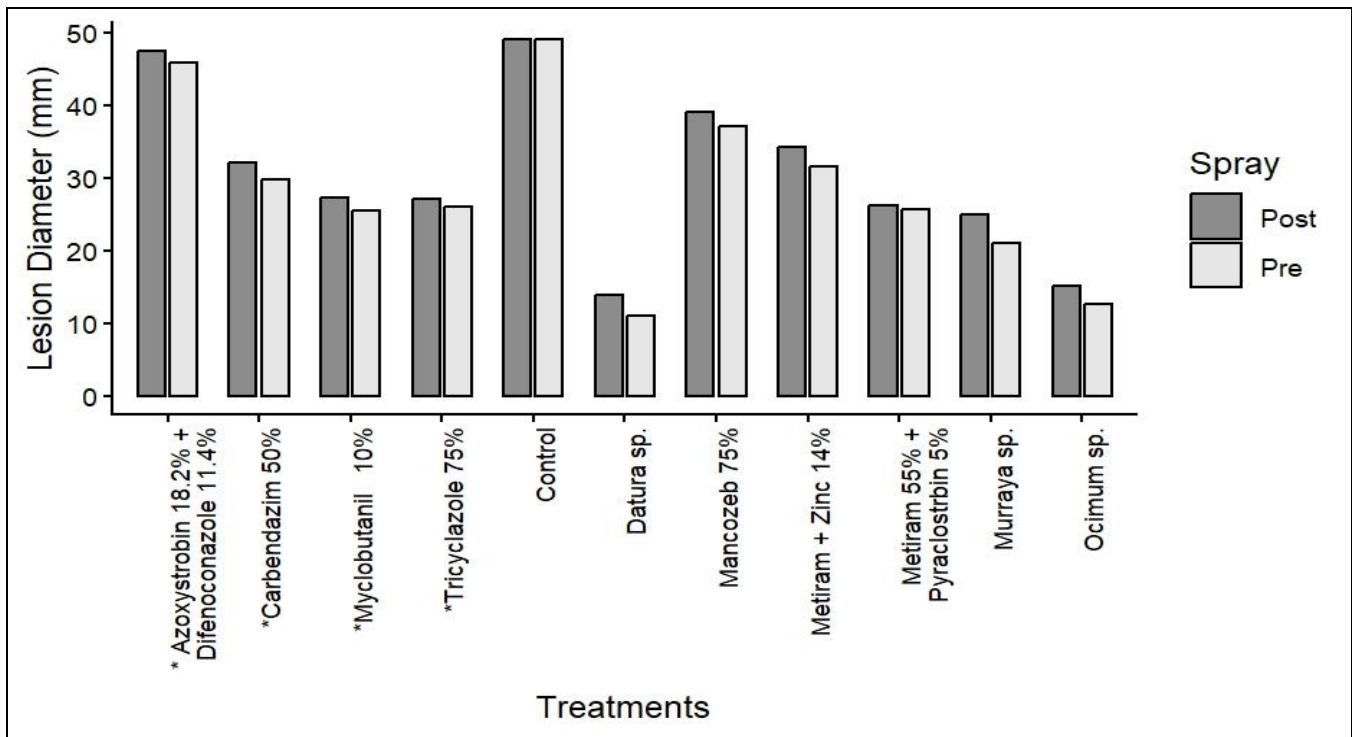
**Fig. 2:** Effect of pre inoculation spray on symptoms development (a) myclobutanil 10% (b) tricyclazole 75% (c) metiram 55% + pyraclostrobin 5% (d) azoxystrobin 18.2% + difenoconazole 11.4% (e) mancozeb 75% (f) metiram + zinc 14% (g) carbendazim 50% (h) *Datura* sp. (i) *Ocimum* sp. (j) *Murraya* sp.



**Fig. 3:** Effect of post inoculation spray on symptoms development (a) myclobutanil 10% (b) tricyclazole 75% (c) metiram 55% + pyraclostrobin 5% (d) azoxystrobin 18.2% + difenoconazole 11.4% (e) mancozeb 75% (f) metiram + zinc 14% (g) carbendazim 50% (h) *Datura* sp. (i) *Ocimum* sp. (j) *Murraya* sp.



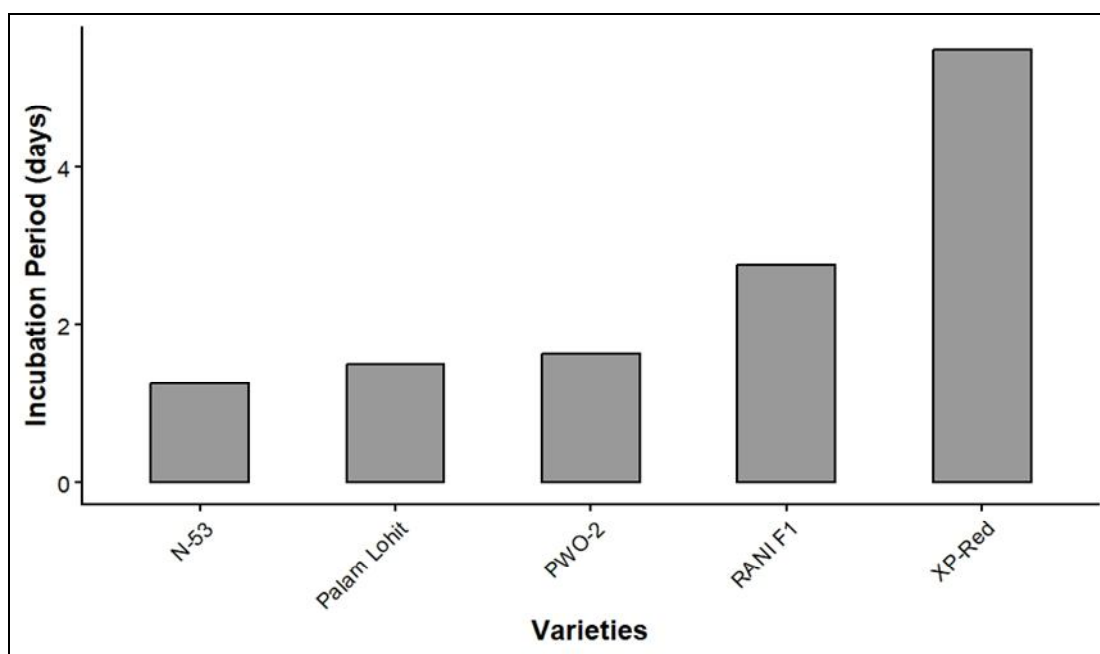
**Fig. 4 :** Effect of Pre and Post Inoculation Spray Treatments on Incubation period in spray (hours)



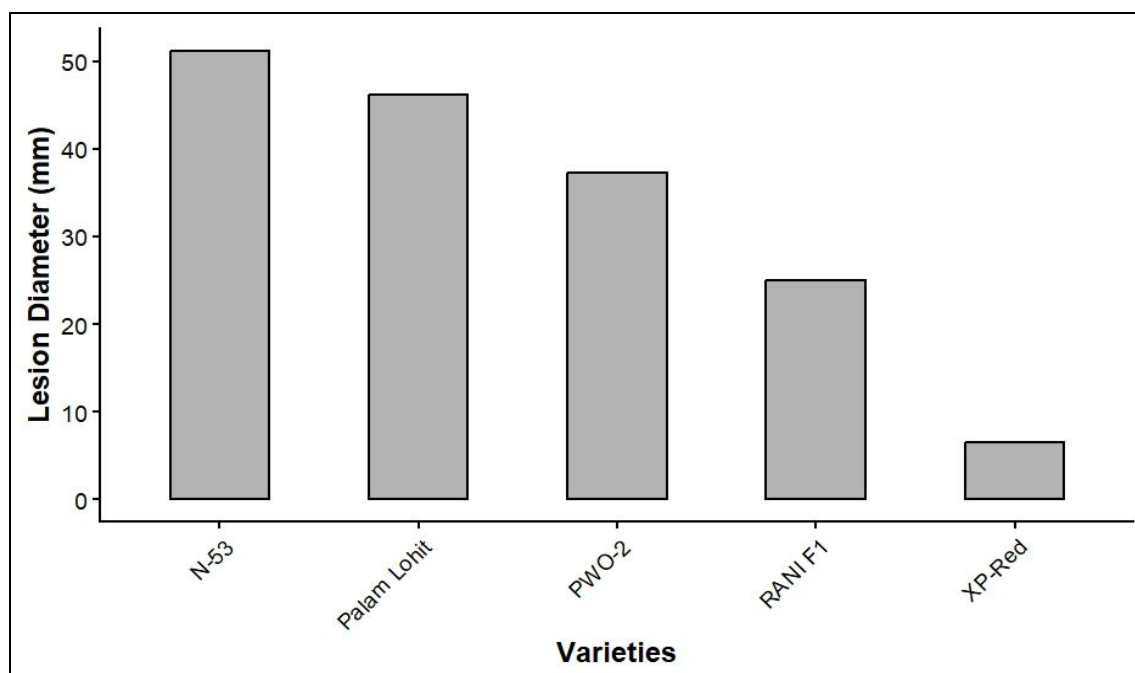
**Fig. 5 :** Effect of Pre and Post Inoculation Spray Treatments on lesion diameter (mm) in spray (hours)



**Fig. 6:** Screening of different varieties against onion smudge (a) PWO-2 (b) N-53 (c) Palam lohit (d) Rani F1 (e) XP Red



**Fig. 7:** Graphical representation of incubation period (days) for screening of different varieties against onion smudge (a) PWO-2 (b) N-53 (c) Palam lohit (d) Rani F1 (e) XP Red



**Fig. 8:** Graphical representation of lesion diameter (mm) for screening of different varieties against onion smudge (a) PWO-2 (b) N-53 (c) Palam lohit (d) Rani F1 (e) XP Red

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### Conflict of Interest

The authors declare that there is no conflict of interest.

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