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Research Article

Management of Purple Blotch Complex of Onion in Indian Punjab

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Abstract

Six systemic fungicides *viz.,* Kitazin 48 EC (iprobenfos), Tilt 25 EC (propiconazole), Folicur 25 EC (tebuconazole), Score 25 EC (difenoconazole), Amistar Top 325 SC (azoxystrobin 18.2% + difenoconazole 11.4%) and Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%), and two non-systemic fungicides *viz.*, Indofil M-45 75 WP (mancozeb) and Kocide 77 WP (copper hydroxide), were evaluated under *in vitro* and field conditions for their efficacy to manage purple blotch complex of onion caused by *Alternaria porri* and *Stemphylium vesicarium*. Field efficacy of the fungicides at different concentrations were determined in controlling the purple blotch complex of onion under artificial epiphytotic conditions on bulb and seed crop (cultivar PRO-6) during the *Rabi* season 2014-2015 and 2015-2016, respectively. The triazole fungicides, tebuconazole and difenoconazole proved superior in inhibiting growth of *A. porri* and *S. vesicarium* under *in vitro* conditions, respectively. Further, foliar sprays (3 for bulb crop and 4 for seed crop) of tebuconazole 25 EC (Folicur) @ 0.1 per cent at fortnightly interval most effectively managed purple blotch complex of onion under field conditions with highest Benefit: Cost ratio (8.75:1 and 88.7:1) in bulb and seed crop, respectively. Seed-to-seed method of onion seed production recorded significantly lower disease severity and higher seed yield than that of bulb-to-seed method under natural epiphytotic conditions. The present findings can be instrumental in devising strategy for the integrated management of *A. porri*, *S. vesicarium* singly as well as in complex, serious limiting biotic factors in onion production.

Keywords: Fungicides; Management; onion; purple blotch complex; Benefit: Cost ratio; trazole

Introduction

Onion (*Allium cepa* L., 2n=16), a bulbous, biennial herb is the most important and one of the five most important fresh market vegetables worldwide (Cramer, 2000). Among vegetables, onion often called as "queen of kitchen" is one of the oldest known and an important crop grown in India. It contains the lachrymatory principle, a strong antibiotic, having fungicidal, bactericidal and nematicidal properties (Purseglove, 1972). It also contains chemical compounds with potential anti-inflammatory, anti-cholesterol, anti-cancer and antioxidant properties, such as quercetin (Slimestad *et al*., 2007). It is also of high medicinal value in controlling human and plant diseases (Vohra *et al.*, 1974).

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India is a traditional producer and assumes second global position in onion production with 19.40 million tonnes from 1.20 million hectares area (Anonymous, 2015). It is cultivated round the year throughout the country. Onion is highly susceptible to many foliar, bulb and root pathogens, which reduce its yield and quality (Cramer, 2000). Among the diseases, purple leaf blotch (PLB) caused by *Alternaria porri* (Ellis) Cif. and *Stemphylium* leaf blight (SLB) caused by *Stemphylium vesicarium* (Wallr.) Simmons, are the major diseases of onion world-wide affecting the foliage severely resulting in crop loss ranging from 30 to 100 per cent both in seed and bulb crop from year to year (Awad *et al.*, 1978; Everts and Lacy, 1990a, b; Brar *et al.*, 1990; Aveling *et al.*, 1993, 1994; Chaput, 1995; Cramer, 2000) and are more prevalent in warm and humid environment (Suheri and Price, 2000a, b).

The low productivity of onion in India is chiefly attributed to prevalence of PLB and SLB in almost all the onion cultivated areas of Northern and Eastern regions (Gupta *et al.*, 1996; Suhag and Bhatia, 2006). Although the PLB and SLB are common on both bulb as well as seed crop of onion, yet it is more damaging particularly in latter, every year in Punjab. The attack is common during March-April coinciding with the bolting of the crop and it may cause 35 to 100 per cent losses (Sharma, 1986). Being weather preferences similar for both PLB and SLB, they mostly occur synchronously on the same umbel bearing stalk resulting additive loss. The typical oval to elliptical lesions were colonised by either *Alternaria porri*, *Stemphylium vesicarium* or mixtures of both pathogens (Suheri and Price, 2001). The lesions containing *A. porri* alone or *S. vesicarium* alone or mixture of both were generally impossible to be differentiated from each other and thus, it was considered to be a complex problem (Suheri and Price 2000a, b). Uddin *et al*. (2006) also reported that the SLB pathogen (*S. vesicarium)* is first to initiate infection, which is followed by subsequent infection by the pathogen of PLB (*A. porri)* and hence, the disease is designated as purple blotch complex (PBC). Resistant cultivars, if available, are the most effective, practical, eco-friendly and economical choice. However, at present, no such resistant varieties are available in India (Shashikanth *et al.*, 2007). Several workers have investigated the potential of conventional as well as new fungicides in different countries of the world for the management of PLB and SLB of onion, individually. However, a perusal of literature revealed that little work appears to have been done on PBC in Punjab and elsewhere. In view of economic importance of the crop, effect of the disease complex on yield and quality and perpetual occurrence of the disease complex , the present study was undertaken to determine *in vivo* and *in vitro* efficacy of novel fungicides and to evaluate the methods of seed production in order to standardize innovative onion purple blotch complex management approaches.

Materials and Methods

Isolation and Purification of the Pathogen Cultures

Isolations of the pathogens were made from the diseased leaf tissue collected from naturally infected onion fields. Typical diseased spot on the leaves were selected and cut into bits of about 1 to 1.5 mm with the help of sterilized scalpel, washed with sterilized distilled water and disinfected with 0.1 percent mercuric chloride ($HgCl_2$) solution for 30 to 60 seconds. These disinfected bits were immediately rinsed in double sterilized distilled water repeatedly to remove the traces of mercuric chloride and toweled on sterilized filter paper, prior to their being aseptically transferred to Petri plates containing 20 ml of autoclaved potato dextrose agar (PDA) in a laminar flow and incubated at 25±1ºC in BOD incubator for 10 days. The resulting fungal culture was purified by hyphal tip technique in PDA slant both for *A. porri* and *S. vesicarium*. The culture of each fungus was further purified by single spore isolation technique.

Cultural and Morphological Studies of the Pathogens

The pathogens isolated in pure form were sub cultured in Petri plates (90 mm diameter) on potato dextrose agar and incubated at 25±1ºC. The observations on colony appearance and growth of the pathogens were recorded for 10 consecutive days.

The ten days old culture of each fungus was used to for morphological characterization and confirmation of identity of isolated pathogens. Identification of the each fungus under study was made after examining 100 conidia under compound microscope (40x). Stage and ocular micrometer were used to measure the length, breadth, beak length and number of septa of the fungus after the calibration of the microscope. The average length and breadth of the conidial body, beak and number of septa were recorded. These observations were compared with those of the standard measurements given by Ellis (1971) and Simmons (1969) to identify the pathogens.

In vitro Evaluation of Different Fungicides against Alternaria porri and Stemphylium vesicarium

Six systemic fungicides *viz.*, Kitazin 48 EC (iprobenfos), Tilt 25 EC (propiconazole), Folicur 25 EC (tebuconazole), Score 25 EC (difenoconazole), Amistar Top 325 SC (azoxystrobin 18.2% + difenoconazole 11.4%) and Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%), and two non-systemic fungicides *viz.*, Indofil M-45 75 WP (mancozeb) and Kocide 77 WP (copper hydroxide), were evaluated for their efficacy against *A. porri* and *S. vesicarium* under *in vitro* conditions following poison food technique after Nene and Thapliyal (1993).

All the systemic fungicides were tested at a series of concentrations *viz.*, 0.5, 1, 10, 25, 50, 100 and 200 µg/ml, respectively while the non-systemic fungicides were tested at a series of concentrations *viz.*, 0.5, 1, 10, 25, 50, 100, 200, 500 and 1000 µg/ml, respectively. The stock solution of the

chemicals prepared in sterilized distilled water was added under aseptic conditions in 250 ml flask containing potato dextrose agar medium so as to have the final concentrations (on *a.i.* basis) of 0.5, 1, 10, 25, 50, 100, 200, 500 and 1000 µg/ml. The fungicide amended medium was poured in sterilized Petri plates, allowed to cool and seeded in the centre with 5 mm mycelial disc of actively growing culture of *A. porri* and *S. vesicarium* separately. The Petri plates containing un-amended PDA medium served as check. Each treatment was replicated four times using one Petri plate in each replicate under completely randomized design (CRD). The inoculated Petri plates were wrapped with parafilm to minimize the chances of contamination and incubated at 25±1°C in BOD incubator. The observations on colony diameter of each fungus were recorded after the Petri plates (90 mm diameter) in control were completely filled with the mycelial growth of the respective fungus. The per cent inhibition in mycelial growth was worked out by using the formula devised by Vincent (1947).

$$P_i = \frac{C - T}{C} \times 100$$

where,

P_i= Per cent inhibition in colony growth

C = Colony growth diameter in control

T = Colony growth diameter in treatment

The efficacy of the fungicides was expressed in terms of ED_{50} and ED_{90} values calculated by probit regression analysis. The per cent inhibition at each concentration was transformed to the empirical probit value in point scale as given by Finney (1977). Then, the empirical probit values were regressed with log concentrations using simple linear regression. The constant parameters "a" and "b" were obtained. The ED_{50} and ED_{90} values were calculated using following formula:

$$ED_{50} = \text{Antilog} \frac{5 - a}{b}$$

$$ED_{90} = \text{Antilog} \frac{6.28 - a}{b}$$

In-Vivo Evaluation of Different Fungicides against Onion Purple Blotch Complex in Bulb and Seed Crop

Field efficacy of six systemic fungicides *viz.*, Kitazin 48 EC (iprobenfos), Tilt 25 EC (propiconazole), Folicur 25 EC (tebuconazole), Score 25 EC (difenoconazole) @ 0.1 per cent, Amistar Top 325 SC (azoxystrobin 18.2% + difenoconazole 11.4%) and Nativo 75 WG (trifloxystrobin 25% + tebuconazole 50%) each @ 0.05 per cent, and two non-systemic fungicides *viz.*, Indofil M-45 75 WP (mancozeb) @ 0.3 per cent and Kocide 77 WP (copper hydroxide) @ 0.25 per cent, were determined in controlling the onion PBC under artificial epiphytotic conditions on bulb and seed crop (cv. PRO-6) during the *Rabi* season 2014-2015 and 2015-2016, respectively. The nursery was raised during last week of October and transplanted in last week of December for bulb crop while for seed crop, the nursery was raised during last week of August and transplanted in last week of October. The bulb as well as seed crop was raised in the plot size of 2 x 2 m^2 at a spacing of 15 cm between rows and 10 cm between plants. The field trials were conducted in a randomized block design (RBD) with three replications along with the unsprayed check in the field area, Department of Plant Pathology as per standard agronomic practices, Punjab Agricultural University, Ludhiana (Anonymous 2013). Artificial epiphytotic conditions were created in the field by spraying mixed inoculum (conidial and mycelial bits suspension) of *A. porri* (1 x 10^4 propagules/ml) and *S. vesicarium* (1 x 10^4 propagules /ml) during evening hours in the first and last week of February. Foliar application of fungicides was started as soon as first symptom of the disease was noticed followed by two and three spray at 15 days interval in bulb and seed crop, respectively.

Twenty-five plants from each plot were randomly tagged for disease assessment on the 0 to 5 rating scale given by Sharma (1986) as follows:

Score	Disease description
0	No disease symptom
1	A few spots covering 10 per cent leaf area
2	Several purplish brown patches covering up to 20 per cent of leaf area
3	Several patches with paler outer zone covering up to 40 per cent leaf area
4	Leaf streaks covering up to 75 per cent leaf area or breaking of the leaves from center
5	Complete drying of the leaves or breaking of leaves from center

The observations on disease severity were recorded 10 days after last fungicidal spray and the per cent disease index (PDI) was computed as per the formulae given by Wheeler (1969).

$$PDI = 100 [\sum SNR] [(N) (MDR)]^{-1}$$

where,

$\sum$SNR is the sum of numerical ratings

N is the number of observations

MDR is the maximum disease rating based on 0-5 scale

The percent disease control was also calculated. The data on fresh bulb size (diameter in cm) were recorded on 25 randomly selected plants per plot with the help of digital Vernier caliper while the data on bulb and seed yield (kg or

g per plot) were recorded after harvesting. Fresh bulb and seed yield per hectare was extrapolated in t/ha and q/ha, respectively. The economics of treatments was worked out by considering the prevailing rates of inputs, produce, labour charges and expressed as an incremental Benefit: Cost ratio. The B:C ratio was calculated using formulae

$$\text{Benefit: Cost (B:C) ratio} = \frac{\text{Additional benefits of the produce from each treatment}}{\text{Additional cost of each treatment}}$$

Evaluation of Method of Seed Production against Purple Blotch Complex in Onion

In order to determine the best method of onion seed production with the respect to incidence and severity of PBC, two recommended seed production method *viz.*, Bulb-to-seed and seed-to-seed, on two onion cultivars Punjab Naroya and PRO-6 were tested under natural epiphytotic conditions. The experiment was carried out in Plant pathology Farm, Punjab Agricultural University, Ludhiana during *Rabi* season 2015-2016 in randomized block design with four replications. The plot size of 2 x 2 m^2 was determined for all the replications. The description of each treatment used during the study is given as below:

(i) Seed-to-seed method:

In this method, healthy onion seeds were sown in nursery beds during last week of August 2015 and forty days old seedlings were transplanted in last week of October 2015 at a spacing of 15 cm between rows and 10 cm between plants. For nursery raising, seeds were sown in 20 cm high nursery beds (size 2 x 1.5 m^2) under green house conditions by using 4-5 kg seeds per 200 m^2 for transplanting in an acre as per the Package of Practices for Vegetable Crops, Punjab Agricultural University, Ludhiana (Anonymous 2013).

(ii) Bulb-to-seed method:

In this method, onion seeds were raised from the healthy bulbs produced during the previous year. The bulbs were planted at 60 cm x 45 cm in the first week of November. The required amount of bulbs was worked out and planted (plot size 2 x 2 ^{m2}). The plots were periodically observed for the number of infected plants and the intensity of PBC and disease incidence and per cent disease index (PDI) was computed according to the formula described in Section 3.1 of this Chapter. The data on the disease severity was recorded on 25 plants selected at random in the field following 0-5 rating scale given by Sharma (1986). The data on seed yield of each plot was also recorded in grams and the seed yield per hectare was extrapolated as q/ha.

The plots were periodically observed for the number of infected plants and the intensity of PBC and disease incidence and per cent disease index (PDI) was computed according to the formula described in Section 3.1 of this Chapter. The data on the disease severity was recorded on 25 plants selected at random in the field following 0-5 rating scale given by Sharma (1986). The data on seed yield of each plot was also recorded in grams and the seed yield per hectare was extrapolated as q/ha.

Statistical Analysis

The data of laboratory studies and field experiments were subjected to analysis of variance as per completely randomized design (CRD) and randomized block design (RBD) using statistical analysis software R Studio and the significance of differences between the treatment means were compared using Least Significant Difference (LSD) and Tukey's honest significant difference (HSD) test at 5 per cent level for proper interpretation of results. The percentages used in different experiments were subjected to arcsine transformations. The data on bulb and seed yield were extrapolated and expressed in t/ha and q/ha, respectively.

Results and Discussion

Cultural Studies

The colony growth of *A. porri* appeared creamy white after 3 days of incubation, which eventually changed to greenish grey and finally turned light olivaceous with distinct light green zonations. The colony was found slightly fluffy with light green raised margins in uniform concentric fashion with radial diameter of 87.43 mm on 10th day of incubation period. Shahnaz *et al* (2013) studied the cultural characteristics of different isolates of *A. porri* and recorded that most of the isolates had fluffy growth on potato dextrose agar with colony colour varying from pinkish white through dull orange to olivaceous and black with distinct to diffuse patterns of zonation.

The colony growth of *S. vesicarium* appeared light grey to dirty white after 3 days of incubation, which finally turned greenish brown to brownish grey. The colony was found fluffy, umbonate to raised with filiform margin and appreciable amount of aerial mycelium with radial diameter of 60.95 mm after 10 days of incubation. Chowdhury (2013) reported that the colony colour of *S. vesicarium* varied from greenish brown to dirty white, deep grey to whitish, light grey to whitish, deep greenish white, light grey and dirty white to greenish with elevation varying from umbonate, raised to flat and texture varying from cottony, fluffy to velvety. Arzanlou *et al* (2012) reported that the colonies of *S. vesicarium* on potato carrot agar medium were grey to brownish grey, flat, attaining a diameter of 50 mm after 7 days with sparse aerial mycelium.

Morphological Studies

The microscopic study of *A. porri* revealed that mycelium of the fungus was initially hyaline which later became pale brown to olivaceous brown or smoky blended with purplish tinge. The hypha were septate and irregularly branched measuring 4.52-7.26 µm in thickness. The conidiophores arose singly or in groups, straight or flexuous pale to mid brown, septate and blunt at the tips. Basal cells of the conidiophore were somewhat wider than the tip cells,

measuring 90.41-95.03 µm x 9.01-12.54 µm with 3 to 5 transverse septa with several prominent conidial scars. Conidia were solitary, straight to curved, obclavate, muriform and light to golden brown with body size 46.01-122.30 µm x 15.77-19.32 µm and beak size 39.52-143.61 µm x 2.06-3.46 µm with 3-12 transverse septa and 0-5 longitudinal septa. These findings are in close conformity with those earlier reported by Ellis (1971), Undhad (2009), Singh (2012), Vijayalaxmi *et al* (2012) and Shahnaz *et al* (2013).

The microscopic study of *S. vesicarium* revealed that the mycelium of the fungus was initially sub hyaline, which later turned pale brown to dark brown. The hypha were septate, smooth irregularly branched measuring 4.02-6.13 µm in thickness. Conidophores were scattered, unbranched, straight to flexuous, smooth, septate and pale to mid brown measuring 42.20-49.07 µm x 4.32-6.46 µm. Conidiogenous cells were monoblastic, terminal, swollen and dark brown. Mature conidia were oblong to broadly ellipsoid, densely verrucose, golden brown to olive brown rounded at the ends and were 24.18-38.43 µm x 12.61- 21.18 µm in size with length to width ratios ranging from 1.64-2.43 with 1-3 transverse and 1-4 longitudinal or oblique septa and often constricted at one or more of the septa. These findings are in close conformity with those of Simmons (1969), Ellis (1971), Basallote *et al* (1999) and Arzanlou *et al* (2012) who reported that mature conidia are produced singly on the swollen apex of the conidiophores and are light to dark brown, densely verrucose, oval to ellipsoidal with transverse and longitudinal septa, and measured 25-42 µm x 12-22 µm.

In Vitro Evaluation of Different Fungicides against Alternaria porri

The data presented in Table 1 revealed significant variation in percent colony growth inhibition among the different systemic fungicides at different test concentrations. Tebuconazole 25 EC (Folicur) proved to be the most effective among the tested systemic fungicides as it completely inhibited the colony growth at 25 µg/ml. It was followed by difenoconazole 25 EC (Score) and trifloxystrobin 25% + tebuconazole 50% WG (Nativo) which were found statistically at par with each other by recording 88.93 and 87.37 per cent inhibition in colony growth at 25 µg/ml, respectively. Trifloxystrobin 25% + tebuconazole 50% WG and propiconazole 25 EC (Tilt) completely inhibited the colony growth at 100 µg/ml and 200 µg/ml, respectively (Fig. 1). At 25 µg/ml, the least colony growth inhibition (20.95%) was recorded by iprobenfos 48 EC (Kitazin) followed by azoxystrobin 18.2% + difenoconazole 11.4% SC (Amistar Top), which recorded 74.17 per cent colony growth inhibition. Iprobenfos 48 EC (Kitazin) proved least effective as it only recorded 45.40 per cent colony growth inhibition at higher concentration of 200 µg/ml. The present findings are in broad agreement with Dinakaran *et al*. (2011), Chethana *et al*. (2011) and Wanggikar *et al.* (2014).

Table 1: *In vitro* evaluation of different systemic fungicides against *Alternaria porri*

Systemic fungicides	**Percent inhibition in colony growth at different concentrations (µg/ml)**							**Mean**
	0.5	**1**	**10**	**25**	**50**	**100**	**200**	
Azoxystrobin 18.2% + difenoconazole 11.4%	52.78^{c} (46.57)	64.16^{bc} (53.21)	70.56^{c} (57.13)	74.17^{d} (59.44)	82.89^{c} (65.55)	88.24^{c} (69.92)	90.20^{b} (71.73)	**74.71^{e} (60.51)**
Difenoconazole	62.43^{ab} (52.20)	68.39^{b} (55.82)	78.36^{ab} (62.30)	88.93^{b} (70.60)	94.64^{b} (76.72)	100.00^{a} (89.96)	100.00^{a} (89.96)	**84.68^{b} (71.08)**
Iprobenfos	9.19^{d} (17.59)	12.68^{d} (20.83)	20.22^{d} (26.69)	20.95^{e} (27.22)	23.08^{d} (28.70)	31.50^{d} (34.13)	45.40^{c} (42.34)	**23.29^{f} (28.21)**
Propiconazole	57.78^{bc} (49.46)	61.39^{cd} (51.57)	77.42^{ab} (61.63)	83.44^{c} (65.97)	86.95^{c} (68.80)	93.50^{b} (75.21)	100.00^{a} (89.96)	**80.07^{d} (66.08)**
Tebuconazole	64.56^{a} (53.47)	76.12^{a} (60.72)	81.89^{a} (64.80)	100.00^{a} (89.96)	100.00^{a} (89.96)	100.00^{a} (89.96)	100.00^{a} (89.96)	**88.94^{a} (76.98)**
Trifloxystrobin 25% + tebuconazole 50%	59.12^{ab} (50.26)	65.32^{bc} (53.91)	73.62^{bc} (59.12)	87.37^{b} (69.24)	92.67^{b} (74.81)	100.00^{a} (89.96)	100.00^{a} (89.96)	**82.59^{c} (69.61)**
Mean	**53.17 (46.34)**	**58.86 (50.00)**	**67.31 (55.04)**	**72.59 (61.48)**	**77.73 (65.12)**	**83.56 (71.50)**	**87.62 (75.94)**	

Figures in parentheses represent growth inhibition in arc sine transformed values

Values in columns with different superscripts are significantly different ($p \leq 0.05$) according to Tukey's HSD test.

	LSD ($p \leq 0.05$)	**S.Em±**
Systemic fungicide =	0.85	0.43
Concentration =	0.91	0.46
Systemic fungicide x Concentration =	2.23	1.13

The data presented in Table 2 revealed significant variation in colony growth inhibition among the non-systemic fungicides at different test concentrations. Mancozeb 75 WP (Indofil M-45) was found significantly superior to copper hydroxide 77 WP (Kocide) at all the test concentrations in terms of per cent colony growth inhibition of *A. porri*. Mancozeb recorded higher mean inhibition of 53.38 per cent as compared to copper hydroxide (38.66 %). At 1000 µg/ml, mancozeb recorded only 91.14 per cent colony growth inhibition and thus was found less effective as compared to systemic fungicides, triazole and strobilurin as it did not record complete colony growth inhibition at such a higher concentration (Fig. 1). The present findings are in agreement with Chethana *et al.* (2011) and Wanggikar *et al.* (2014) who reported that mancozeb was the most effective fungicide against *A. porri* under *in vitro* condition, among the non-systemic fungicides.

The data (Table 3) revealed that tebuconazole had the lowest ED_{50} value (0.22 µg/ml) followed by difenoconazole (0.28 µg/ml), trifloxystrobin 25% + tebuconazole 50% (0.35 µg/ml), propiconazole (0.36 µg/ml), azoxystrobin 18.2% + difenoconazole 11.4% (0.47 µg/ml), mancozeb (52.29 µg/ml), copper hydroxide (115.90 µg/ml) and iprobenfos (214.64 µg/ml). Similarly, tebuconazole recorded the lowest ED_{90} value (16.72 µg/ml) followed by difenoconazole (35.05 µg/ml), trifloxystrobin 25% + tebuconazole 50% (41.85 µg/ml), propiconazole (72.04 µg/ml), azoxystrobin 18.2% + difenoconazole 11.4% (157.97 µg/ml), mancozeb (768.28 µg/ml), copper hydroxide (902.94 µg/ml) and iprobenfos (1278.39 µg/ml), respectively. Iprobenfos recorded the highest ED_{50} and ED_{90} values and thus proved to be ineffective for inhibiting mycelial growth of *A. porri*.

Table 2: *In vitro* evaluation of different non-systemic fungicides against *Alternaria porri*

Non-systemic fungicides	**Percent inhibition in colony growth at different concentrations (µg/ml)**									**Mean**
	0.5	**1**	**10**	**25**	**50**	**100**	**200**	**500**	**1000**	
Copper hydroxide	9.09[b] (17.47)	18.89[b] (25.61)	22.92[b] (28.59)	24.31[b] (29.53)	28.72[b] (32.39)	41.33[b] (39.93)	59.78[b] (50.64)	62.54[b] (52.25)	80.39[b] (63.79)	**38.66[b] (42.44)**
Mancozeb	9.39[a] (17.80)	29.39[a] (32.81)	38.54[a] (38.36)	45.75[a] (42.54)	48.22[a] (43.96)	56.77[a] (48.87)	78.16[a] (62.12)	83.06[a] (65.67)	91.14[a] (72.66)	**53.38[a] (53.45)**
Mean	**9.24 (17.64)**	**24.14 (29.21)**	**30.73 (33.47)**	**35.03 (36.03)**	**38.47 (38.18)**	**49.05 (44.40)**	**68.97 (56.38)**	**72.80 (58.96)**	**85.76 (68.22)**	-

Figures in parentheses represent growth inhibition in arc sine transformed values
Values in columns with different superscripts are significantly different ($p \leq 0.05$) according to Tukey's HSD test.

	LSD ($p \leq 0.05$)	**S.Em±**
Non-Systemic fungicide =	0.89	0.44
Concentration =	1.89	0.94
Non-Systemic fungicide x Concentration =	2.67	1.33

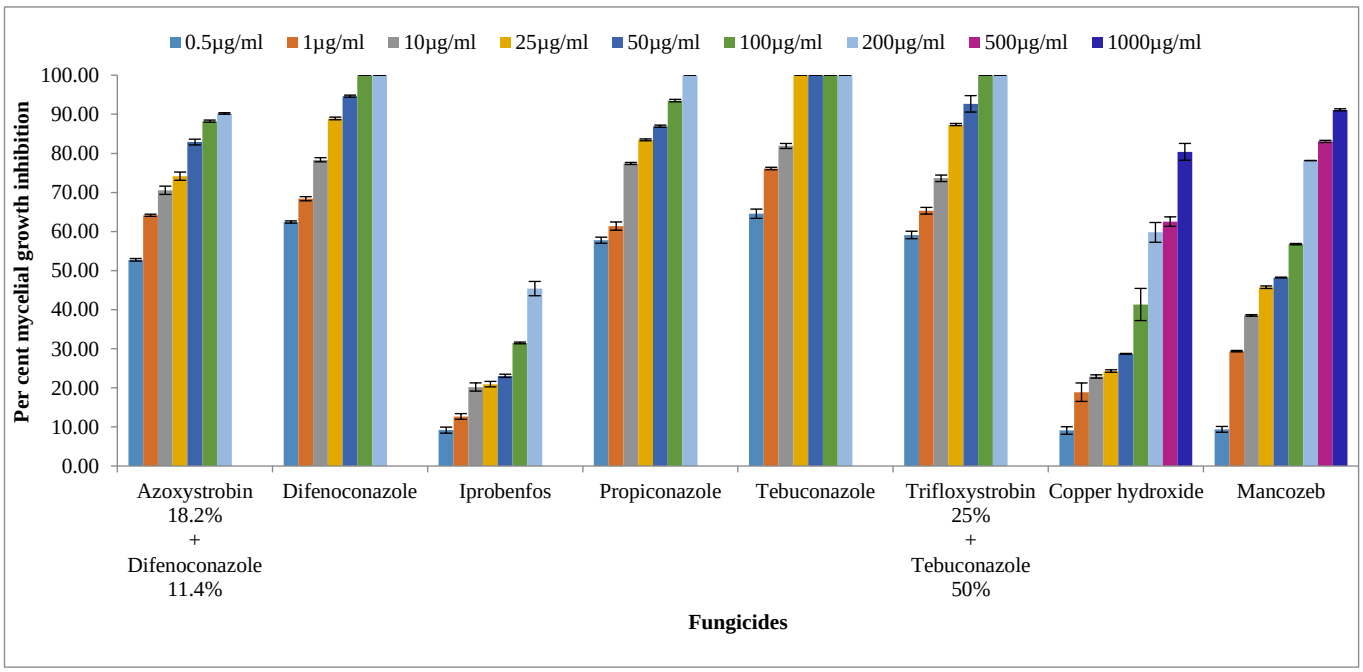

Fig. 1: Percent inhibition in colony growth of *Alternaria porri* with different fungicides at their respective concentrations

Table 3: ED_{50} and ED_{90} values of different fungicides against *Alternaria porri*

Fungicides	ED values (µg/ml)	
	ED_{50}	**ED_{90}**
Azoxystrobin 18.2% + difenoconazole 11.4%	0.47	157.97
Copper hydroxide	115.90	902.94
Difenoconazole	0.28	35.05
Iprobenfos	214.64	1278.39
Mancozeb	52.29	768.28
Propiconazole	0.36	72.04
Tebuconazole	0.22	16.72
Trifloxystrobin 25% + tebuconazole 50%	0.35	41.85

In Vitro Evaluation of Different Fungicides against Stemphylium vesicarium

The data presented in Table 4 revealed significant variation in colony growth inhibition among the different systemic fungicides at different test concentrations. Difenoconazole 25 EC (Score) was the most effective among the tested systemic fungicides as it completely inhibited colony growth at 10 µg/ml. It was followed by azoxystrobin 18.2% + difenoconazole 11.4% SC (Amistar Top), propiconazole 25 EC (Tilt 25) and tebuconazole 25 EC (Folicur) which recorded 73.26, 71.14 and 63.06 per cent colony growth inhibition at 10 µg/ml. Azoxystrobin 18.2% + difenoconazole 11.4% SC and propiconazole 25 EC were found statistically at par with each other. At 200 µg/ml, azoxystrobin 18.2% + difenoconazole 11.4% SC completely inhibited the colony growth of the fungus (Fig. 2). Iprobenfos 48 EC (Kitazin) was found to be least effective at lower concentrations. At higher concentration (200 µg/ml) it, however, recorded 62.79 per cent colony growth inhibition, which is too high a concentration for a systemic fungicide, which is normally used at 50 µg/ml or so. Barnwal (2003) reported that hexaconazole (0.1%) recorded the highest colony growth inhibition of *S. vesicarium* of 95.14 per cent among the six fungicides *viz.*, thiram, hexaconazole, copper oxychloride, benomyl, mancozeb and thiophanate methyl. Hosen (2011) reported that iprodione was found to be the most effective fungicide to inhibit the radial mycelial growth of *Botrytis cinerea* and *S. botryosum* as compared to carbendazim and mancozeb 64 + metalaxyl 8 WG. In a growth chamber experiment to evaluate the efficacy of various fungicides against *S. vesicarium*, tebuconazole 25 EC (Folicur) @ 0.25 ml *a.i.* l^{-1} and procymidone 50 WP (Salithiex) @ 0.5 g *a.i.* l^{-1} reduced the disease severity by 90 per cent while fosetyl-Al 80 WP (Alliete) @ 2.0 g *a.i.* l^{-1} reduced the disease by 75 per cent in garlic (Basallote-Ureba *et al.*, 1998).

The data presented in Table 5 revealed significant variation in colony growth inhibition among the different non-systemic fungicides at different test concentrations. At 0.5 µg/ml, copper hydroxide 77 WP gave non-significant effect when compared to un-amended controls in terms of per cent colony growth inhibition. Mancozeb 75 WP (Indofil M-45) was found significantly superior to copper hydroxide 77 WP (Kocide) at all the test concentrations and recorded higher mean colony growth inhibition of 45.70 per cent as compared to copper hydroxide (32.01%). At 1000 µg/ml, mancozeb recorded only 74.44 per cent colony growth inhibition and thus was found less effective when compared to systemic triazole and strobilurin fungicides, as it did not record complete colony growth inhibition at such a higher concentration. Singh and Milne (1974) reported that mancozeb exhibited high fungicidal activity at or below 100 µg/ml a.i. against A. alternata and S. vesicarium, while all the other fungicides viz., bavistin, benomyl, captafol, captan, carboxin, chloroneb, chlorothalonil, dicloran, fuberidazole, oxycarboxin, thiabendazole, thiophanate methyl, thiram and zineb were not effective. Mishra and Gupta (2012) also reported that mancozeb @ 0.2 per cent was most effective in inhibiting the mycelial growth of S. vesicarium.

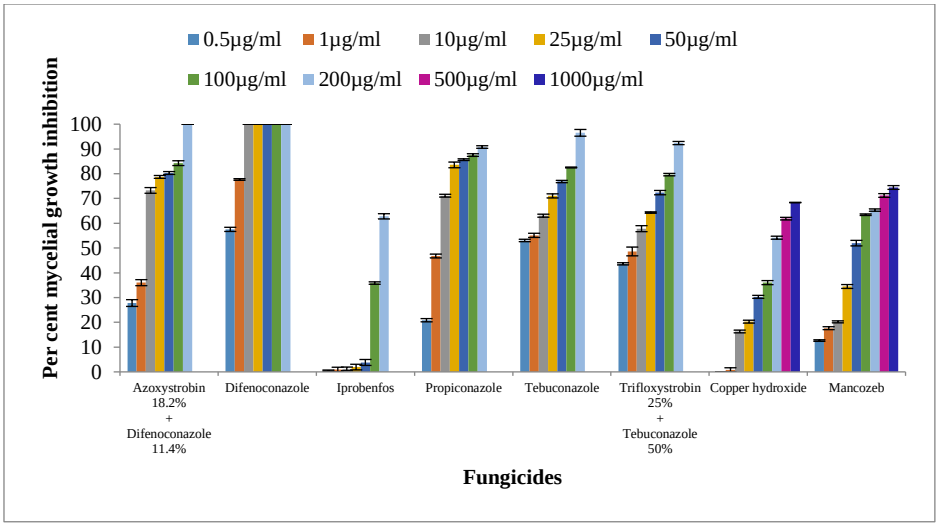

Fig. 2: Percent inhibition in colony growth of *Stemphylium vesicarium* with different fungicides at their respective concentrations

Table 4: *In vitro* evaluation of different systemic fungicides against *Stemphylium vesicarium*

Systemic fungicides	Percent inhibition in colony growth at different concentrations (µg/ml)							Mean
	0.5	1	10	25	50	100	200	
Azoxystrobin 18.2% + difenoconazole 11.4%	27.79^{c} (31.74)	36.02^{c} (36.84)	73.26^{b} (58.95)	78.71^{bc} (62.50)	80.29^{bc} (63.63)	84.26^{bc} (66.63)	100.00^{a} (89.96)	**68.62^{b} (58.61)**
Difenoconazole	57.53^{a} (49.31)	77.67^{a} (61.85)	100.00^{a} (89.96)	100.00^{a} (89.96)	100.00^{a} (89.96)	100.00^{a} (89.96)	100.00^{a} (89.96)	**90.74^{a} (80.14)**
Iprobenfos	0.47^{d} (2.78)	0.94^{d} (2.80)	1.22^{e} (4.47)	1.94^{e} (5.67)	3.81^{d} (8.00)	35.86^{d} (36.77)	62.79^{c} (52.42)	**15.29^{d} (16.13)**
Propiconazole	20.86^{c} (27.11)	46.78^{b} (43.13)	71.14^{bc} (57.49)	83.56^{b} (66.09)	85.67^{b} (67.73)	87.53^{b} (69.30)	90.78^{b} (72.31)	**69.47^{b} (57.59)**
Tebuconazole	53.06^{ab} (46.74)	55.12^{b} (47.92)	63.06^{cd} (52.55)	71.03^{cd} (57.42)	76.85^{c} (61.22)	82.51^{bc} (65.25)	96.51^{b} (74.27)	**71.16^{b} (57.91)**
Trifloxystrobin 25% + tebuconazole 50%	43.62^{b} (41.32)	48.62^{b} (44.19)	57.82^{d} (49.49)	64.32^{d} (53.32)	72.37^{c} (58.32)	79.63^{c} (63.26)	92.38^{b} (74.81)	**65.54^{c} (54.96)**
Mean	**33.89 (33.17)**	**44.19 (39.46)**	**61.08 (52.15)**	**66.59 (55.83)**	**69.83 (58.14)**	**78.30 (65.19)**	**90.41 (75.62)**	

Figures in parentheses represent growth inhibition in arc sine transformed values
Values in columns with different superscripts are significantly different ($p \leq 0.05$) according to Tukey's HSD test

	LSD ($p \leq 0.05$)	S.Em±
Systemic fungicide =	1.52	0.77
Concentration =	1.64	0.83
Systemic fungicide x Concentration =	4.04	2.04

Table 5: *In vitro* evaluation of different non-systemic fungicides against *Stemphylium vesicarium*

Non-Systemic fungicides	Percent inhibition in colony growth at different concentrations (µg/ml)									
	0.5	1	10	25	50	100	200	500	1000	Mean
Copper hydroxide	0.00^{b} (0.00)	0.83^{b} (2.63)	16.31^{b} (23.80)	20.29^{b} (26.76)	30.29^{b} (33.37)	36.04^{b} (36.87)	54.18^{b} (47.38)	61.86^{b} (51.63)	68.36^{b} (56.71)	**32.01^{b} (31.02)**
Mancozeb	12.64^{a} (20.75)	17.64^{a} (24.82)	20.19^{a} (26.69)	34.47^{a} (35.92)	51.97^{a} (46.11)	63.44^{a} (52.78)	65.31^{a} (53.89)	71.22^{a} (57.54)	74.44^{a} (59.62)	**45.7^{a} (42.01)**
Mean	**6.32 (10.37)**	**9.24 (13.72)**	**18.25 (25.24)**	**27.38 (31.34)**	**41.13 (39.74)**	**49.74 (44.83)**	**59.75 (50.63)**	**66.54 (54.59)**	**71.40 (58.17)**	-

Figures in parentheses represent growth inhibition in arc sine transformed value
Values in columns with different superscripts are significantly different ($p \leq 0.05$) according to Tukey's HSD test

	LSD ($p \leq 0.05$)	S.Em±
Non-systemic fungicide=	0.78	0.39
Concentration =	1.66	0.83
Non-systemic fungicide x Concentration =	2.34	1.17

The data (Table 6) revealed that difenoconazole recorded the lowest ED_{50} value (0.43 µg/ml) followed by tebuconazole (0.46 µg/ml), propiconazole (3.24 µg/ml), trifloxystrobin 25% + tebuconazole 50 per cent (3.28 µg/ml), azoxystrobin 18.2% + difenoconazole 11.4% (3.63 µg/ml), mancozeb (42.60 µg/ml), iprobenfos (162.81 µg/ml) and copper hydroxide (196.52 µg/ml), respectively. Similarly, difenoconazole recorded the lowest ED_{90} value (3.99 µg/ml) followed by azoxystrobin 18.2% + difenoconazole 11.4% (114.41 µg/ml), tebuconazole (131.26 µg/ml), propiconazole (180.99 µg/ml), trifloxystrobin 25% + tebuconazole 50% (186.37), iprobenfos (248.95 µg/ml), mancozeb (1363.40 µg/ml) and copper hydroxide (1601.02 µg/ml), respectively.

Table 6: ED_{50} and ED_{90} values of different fungicides against *Stemphylium vesicarium*

Fungicides	ED values (µg/ml)	
	ED_{50}	ED_{90}
Azoxystrobin 18.2% + difenoconazole	3.63	114.41
Copper hydroxide	196.52	1601.02
Difenoconazole	0.43	3.99
Iprobenfos	162.81	248.95
Mancozeb	42.60	1363.40
Propiconazole	3.24	180.99
Tebuconazole	0.46	131.26
Trifloxystrobin 25% + tebuconazole 50%	3.28	186.37

In vivo evaluation of different fungicides against onion purple blotch complex (PBC) in bulb and seed crop

The data presented in Table 7 and Table 8 revealed that all the fungicidal treatments were significantly superior to the untreated check in reducing the disease severity and increasing the bulb and seed yield. The analysis of variance showed significant variation in the disease severity and yield parameters among the different fungicidal treatments in both bulb and seed crops. Systemic triazole and strobilurin fungicides were found more effective as compared to non-systemic fungicides.

Tebuconazole 25 EC (Folicur) @ 0.1 per cent was found to be the most effective in reducing the disease severity (21.53 and 28.37%) which was followed by trifloxystrobin 25% + tebuconazole 50% WG (Nativo) @ 0.05 per cent (26.95 and 31.83%), difenoconazole 25 EC (Score) @ 0.1 per cent (34.01 and 37.03%), azoxystrobin 18.2% + difenoconazole 11.4% SC (Amistar Top) @ 0.05 per cent (42.66 and 44.26%), propiconazole 25 EC (Tilt 25) @ 0.1 per cent (46.85 and 46.40%) and mancozeb 75 WP (Indofil M-45) @ 0.3 per cent (52.90 and 52.97%) as compared to 73.68 and 82.36 per cent disease severity in untreated control in bulb and seed crop, respectively. Iprobenfos 48 EC (Kitazin) @ 0.1 per cent was found least effective as it recorded the highest disease severity (68.46 and 77.74%). This was followed by copper hydroxide 77 WP (Kocide) @ 0.25 per cent (63.67 and 68.32%) in bulb and seed crop, respectively. Highest disease control was recorded in foliar application of tebuconazole 25 EC (70.78 and 65.55%) which was followed by trifloxystrobin 25% + tebuconazole 50% WG (63.42 and 61.35%) and difenoconazole 25 EC (53.84 and 55.04%) in bulb and seed crop.

Highest fresh bulb and seed yield were recorded in trifloxystrobin 25% + tebuconazole 50% WG (18.54 t/ha and 3.34 q/ha) followed by tebuconazole 25 EC (18.12 t/ha and 3.12 q/ha) and difenoconazole 25 EC (17.64 t/ha and 2.85 q/ha), respectively. Similarly, highest fresh bulb size was recorded in trifloxystrobin 25% + tebuconazole 50% WG (5.56 cm) followed by tebuconazole 25 EC (5.51 cm) and difenoconazole 25 EC (5.43 cm), respectively. Trifloxystrobin 25% + tebuconazole 50% WG and tebuconazole 25 EC were found statistically at par with respect to fresh bulb as well as seed yield and size of fresh bulbs. All the fungicidal treatments resulted in significant increase in benefit: cost (B:C) ratio. The highest B:C ratio was recorded in tebuconazole (8.75:1 and 88.67:1) while the lowest B:C ratio was recorded in iprobenfos (0.46:1 and 3.41:1) in bulb and seed crop, respectively. The present findings revealed that despite of significantly higher disease control recorded by tebuconazole in bulb and seed crop, trifloxystrobin 25% + tebuconazole 50% WG (Nativo) was found to record numerically higher fresh bulb yield as well as size and seed yield. It could be attributed to growth promoting effects of azoxystrobin (a strobilurin compound) present in the commercial formulation.

Table 7: *In vivo* evaluation of different fungicides against onion purple blotch complex in bulb crop during *Rabi* 2014-15

Fungicides	**Concentration (%)**	**PDI***	**PDC****	**Fresh bulb Yield (t/ha)**	**Fresh bulb size (cm)**	**Benefit: cost ratio**
Azoxystrobin 18.2 + difenoconazole 11.4 SC	0.05	42.66^{e} (40.76)	42.10	17.10^{c}	5.34^{c}	6.54:1
Copper hydroxide 77 WP	0.25	63.67^{c} (52.94)	13.59	13.45^{f}	4.47^{f}	0.59:1
Difenoconazole 25 EC	0.1	34.01^{f} (35.65)	53.84	17.64^{b}	5.43^{bc}	7.95:1
Iprobenfos 48 EC	0.1	68.46^{b} (55.86)	7.08	13.06^{fg}	4.34^{g}	0.46:1
Mancozeb 75 WP	0.3	52.90^{d} (46.65)	28.20	14.48^{e}	4.73^{e}	3.58:1
Propiconazole 25 EC	0.1	46.85^{e} (43.17)	36.41	15.27^{d}	4.98^{d}	5.33:1
Tebuconazole 25 EC	0.1	21.53^{h} (27.62)	70.78	18.12^{ab}	5.51^{ab}	8.75:1
Trifloxystrobin 25 + tebuconazole 50 WG	0.05	26.95^{g} (31.25)	63.42	18.54^{a}	5.56^{a}	6.87:1
Untreated control	-	73.68^{a} (59.19)	-	12.87^{g}	4.27^{g}	-
SE.m±	-	0.69	-	0.14	-	-
LSD (p≤0.05)	-	1.47	-	0.26	-	-

*Per cent disease index

** Per cent disease control

Figures in parentheses represent per cent disease index in arc sine transformed value

Values in the column with different superscripts are significantly different (p≤0.05) according to Tukey's HSD test

Table 8: *In vivo* evaluation of different fungicides against onion purple blotch complex in seed crop during *Rabi* 2015-16

Fungicides	Concentration (%)	PDI*	PDC**	Seed yield(q/ha)	Increase in seed yield over control (%)	Benefit:cost ratio
Azoxystrobin 18.2 + difenoconazole 11.4 SC	0.05	44.26^{e} (41.68)	46.26	2.47^{c}	190.59	58.70:1
Copper hydroxide 77 WP	0.25	68.32^{c} (55.76)	17.05	1.06^{e}	24.71	5.05:1
Difenoconazole 25 EC	0.10	37.03^{f} (37.46)	55.04	2.85^{b}	235.29	78.13:1
Iprobenfos 48 EC	0.10	77.74^{b} (61.92)	5.61	0.91^{e}	7.06	3.41:1
Mancozeb 75 WP	0.30	52.97^{d} (46.69)	35.68	1.78^{d}	109.41	48.44:1
Propiconazole 25 EC	0.10	46.40^{e} (42.91)	43.66	2.36^{c}	177.65	78.65:1
Tebuconazole 25 EC	0.10	28.37^{g} (32.16)	65.55	3.12^{ab}	267.06	88.67:1
Trifloxystrobin 25 + tebuconazole 50 WG	0.05	31.83^{g} (34.32)	61.35	3.34^{a}	292.94	70.74:1
Untreated control	-	82.36^{a} (65.30)	-	0.85^{e}	-	-
S.Em±	-	0.83	-	0.09	-	-
LSD (p≤0.05)	-	1.77	-	0.19	-	-

*Percent disease index
** Percent disease control
Figures in parentheses represent percent disease index in arc sine transformed value
Values in the column with different superscripts are significantly different (p≤0.05) according to Tukey's HSD test.

Table 10: Evaluation of method of seed production against purple blotch complex in onion during *Rabi* 2015-16

Cultivars	Bulb-to-seed method			Seed-to-seed method			Mean PDI	Mean seed yield (q/ha)
	DI**	PDI*	Seed yield (q/ha)	PDI	DI	Seed yield (q/ha)		
PRO-6	100	60.16 (50.86)	0.86	100	53.54 (47.02)	1.82	**56.85 (48.94)**	**1.34**
Punjab Naroya	100	74.21 (59.53)	0.60	100	61.59 (51.72)	1.43	**67.90 (55.63)**	**1.02**
Mean	**100**	**67.19 (55.20)**	**0.73**	**100**	**57.56 (49.37)**	**1.62**	-	-

Figures in parentheses represent per cent disease index in arc sine transformed value
*Per cent disease index
**Per cent disease incidence

Source of variance	PDI		Seed Yield	
	S.Em±	LSD(p≤0.05)	S.Em±	LSD(p≤0.05)
Variety (V)	0.70	1.58	0.04	0.09
Method of seed production (M)	0.70	1.58	0.04	0.09
V x M	0.99	2.25	0.06	0.13

The present findings are in close agreement with those earlier reported by Upamanyu (1999), Deshmukh *et al.* (2007), Beig *et al.* (2008), Aujila *et al.* (2010), Aujila *et al.* (2013) and Bhatia and Chahal (2014) who have also reported the superiority of tebuconazole, propiconazole, difenoconazole and hexaconazole over conventional fungicides in controlling the disease. Similarly, the present findings are also in agreement with earlier reported by Vincelli (2002), Nelson and Meinhardt (2011), Henry *et al.* (2011) and Hill *et al.* (2013) who have reported the growth promoting effects of strobilurins in several plant species.

Evaluation of Method of Seed Production against Purple Blotch Complex in Onion

The data presented in Table 9 revealed significant variation among cultivars and different methods of seed production in terms of per cent disease index (PDI) and seed yield. However, no difference in disease incidence was recorded among the different cultivars and methods of seed production of onion. Significantly higher mean PDI (67.19%) was recorded in bulb-to-seed method as compared to mean PDI of 57.56 per cent in seed-to-seed method. Similarly, significantly higher mean PDI (67.90%) was recorded in cultivar Punjab Naroya as compared to mean PDI of 56.85 per cent in cultivar PRO-6. Significantly higher mean seed yield (1.62 q/ha) was recorded in seed-to-

seed method as compared to 0.73 q/ha in bulb-to-seed method. Similarly, significantly higher mean seed yield (1.34 q/ha) was recorded by cultivar PRO-6 as compared to 1.02 q/ha recorded on cultivar Punjab Naroya. It is evident from the data presented in Table 9 that the seed-to-seed method using cultivar PRO-6 recorded lower disease severity and higher seed yield as compared to other treatments. As the literature is silent regarding the effect of methods of seed production on disease incidence and severity, the present findings remain uncompared and new.

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