Plant Pathology & Quarantine 10(1): 198–216 (2020) ISSN 2229-2217
www.ppqjournal.org **Article**

Doi 10.5943/ppq/10/1/19

Chemical Management of Anthracnose-Twister (*Colletotrichum gloeosporioides* and *Fusarium fujikuroi*) Disease of Onion (*Allium cepa*)

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Perez PM, Alberto RT 2020 – Chemical Management of Anthracnose-Twister (*Colletotrichum gloeosporioides* and *Fusarium fujikuroi*) Disease of Onion (*Allium cepa*) Plant Pathology & Quarantine 10(1), 198–216, Doi 10.5943/ppq/10/1/19

Abstract

Among all other diseases of onion, anthracnose-twister caused by *Colletotrichum gloeosporioides* and *Fusarium fujikuroi* (formerly known as *Gibberella moniliformis*) remained the most destructive every cropping season. It destroys onion production, especially when left unmanaged. Studies showed that chemical management is still the best option in addressing this disease; thus, this study was conducted. It aimed to: (a) evaluate the different protectant fungicides available in the market in managing the disease; (b) test Paclobutrazol in suppressing the rapid production of gibberellic acid that triggers the development of the disease; and (c) compare the performance of Paclobutrazol and other fungicides in suppressing the disease when applied as preventative and curative. Results showed that onion plants treated with Captan and Paclobutrazol had the least incidence of disease under protective spray application. In contrast, onion plants applied with Carbendazim and Paclobutrazol had the least severity on both spray applications. Whereas, onion plants applied with Difenoconazole-Propiconazole and Auxin showed the highest disease incidence and severity in both spray applications. The shortest length of the neck was observed in onion plants treated with Benomyl+Paclobutrazol and Paclobutrazol alone, while the longest was observed in Difenoconazole-Propiconazole. The highest yield was obtained in onion plants treated with Carbendazim+Paclobutrazol and Captan, whereas the lowest yield was obtained in onions applied with Auxin and Difenoconazole-Propiconazole. Protective spray application obviously showed lower disease incidence and severity, shorter neck and higher yield compared to curative spray application.

Key words – Anthracnose-Twister Disease – Disease Complex – Chemical Management

Introduction

Onion has been one of the high valued crops grown in the Philippines and its productivity significantly contributes an immense increase to the economies income. However, according to Alberto et al. (2002), its production is at a decreasing trend from 1996-2000 resulting in low supply and high cost in the local market as well as limited quantities of onion for export. Onion production

Submitted 17 July 2020, Accepted 12 November 2020, Published 26 November 2020
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went down further by 2.95% in this period (BAS 2012). One of the various factors that contributed to low production are pests and diseases (Alberto et al. 2002) as well as unfavourable weather conditions which affected the plant's growth and development and late detection of anthracnose (BAS 2012). Among the diseases, the anthracnose-twister plays a vital role in its low productivity which at present is considered to be the most destructive disease of onion in the country (Alberto et al. 2002). This has been the most destructive disease of onion reported since 2001 until today. Development of novel ways to address the losses and decreased production of onion in the Philippines due to diseases has been of great interest since 1996 (Alberto 2014). Chemical management would be the most effective and promising approach to be used under the manufacturer's recommended rate as it could address this destructive disease immediately; thus this study was conducted.

Materials & Methods

Pathogenicity Test

Onion plants infected with "anthracnose-twister" disease were collected from onion growing areas in Bongabon, Nueva Ecija, Philippines. The two target pathogens namely: *Colletotrichum gloeosporioides* and *Fusarium fujikuroi* were isolated from the infected yellow onion plant variety showing obvious symptoms like the presence of sunken lesion with orange to black masses of mycelia on the leaves and twisting from the neck. It was then subjected to pure culture production and afterwards, mass produced. Subsequently, it was once tested for its pathogenicity to the onion cultivar where it was isolated. Ten test plant samples per pathogen were used. Before inoculation, the spore densities of the two pathogens were standardized at 2.5×10^6 before 25 µl of the fungal suspensions were dropped in four inoculation points i.e. two in the leaf and two in the neck. The host plants were observed for symptom development. Koch's Postulates were satisfied after the pathogens were re-isolated from the host plants and re-checked for morphological characteristics.

Pot Experiment

Sixty (60) day old onion seedlings (c.v. Yellow Granex) were grown in pots (one plant/ pot). Fourteen-day old sporulating cultures of the pathogens were flooded with 100 mL sterile distilled water and the surface growth was scraped. The spore suspension was filtered through four layers of cheesecloth to separate the mycelia from the spores. The spore densities (2.5×10^6) for both pathogens were adjusted using a haemocytometer. The potted onion plants were divided into two components. The first component was Curative, and the second component was Protective. Each component was further subdivided into three sets; which were made up of forty-four potted onion plants in each set as follows:

Set 1 – Inoculated with *Fusarium fujikuroi*
Set 2 – Inoculated with *Colletotrichum gloeosporioides*
Set 3 – Inoculated with *Colletotrichum gloeosporioides* and *Fusarium fujikuroi*

The plants in the first component (Curative) were spray inoculated with fungal suspension and immediately covered with a plastic bag for overnight incubation to maintain high relative humidity. The plastic covers were removed early in the morning to prevent the plant from heat stress and put in place again late in the afternoon. High relative humidity was maintained during the day thru mist application three times in a day (i.e. 9 AM, 12Noon and 3 PM) for three consecutive days. Plants were covered with a plastic bag for three straight nights for symptom development.

Six (6) days after inoculation, the four most promising fungicides in the previously conducted in-vitro experiments were used together with Paclobutrazol as one of the treatments in the infected potted onion plants (Tables 1, 2).

Table 1 Chemical treatments used in pot experiment

Treatment	Chemical
T1	Captan (CAP)
T2	Benomyl (BEN)
T3	Difenoconazole-Propiconazole (DP)
T4	Carbendazim (CAR)
T5	Paclobutrazol (PAC)
T6	Captan+Paclobutrazol (CAP+PAC)
T7	Benomyl+Paclobutrazol (BEN+PAC)
T8	Difenoconazole-Propiconazole+Paclobutrazol (DP+PAC)
T9	Carbendazim+Paclobutrazol (CAR+PAC)
T10	Positive Control (Inoculated)
T11	Negative Control (Uninoculated)
T12	Auxin

Table 2 Commercial chemicals used in the pot experiment

Product name	Active ingredients	Recommended rate	Manufacturer
Captan 50WP	Captan (500g/kg)	4-8tbsp./16L	Arvesta
Venom 50WP	Benomyl (500g/kg)	1-2tbsp./16L	Kajo Agro-Chemical Company
Armure 300EC	Difenoconazole (150g/kg) Propiconazole (150g/kg)	1.5-2.0tbsp./16L	Syngenta Phil. Inc
Bavistin 50DF	Carbendazim (500g/kg)	1-2tbsp./16L	BASF SE
Steady 10WP	(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H,2,4-triazol-1-yl=pentan-3-ol (100g/kg)	80g/16L	Syngenta Phil. Inc

The chemical treatments were sprayed on the surface of the previously inoculated leaves using hand-held sprayer, whereas the paclobutrazol was applied in the soil by soil drenching following manufacturer's recommended rate. Sterile distilled water was used in the untreated control plants. Plants were placed in an elevated platform inside the screen house at temperature ranging from 28^{o}C-29^{o}C. The set up was arranged in CRD with four (4) replications. Plants were maintained based on onion's recommended cultural management practices, and the insect pests were managed through hand picking. The data were analyzed by ANOVA using SAS v.9.1 software. Critical differences among treatments were calculated at 5% probability level of significance using Tukey's Studentized Range (HSD) Test.

Disease evaluation was carried out a day before the next spraying schedule and treatments were applied six times at 7 days interval for a total of 6 treatment applications. Disease development on the leaves was scored on 0-9 scale at 7 days after inoculation Tables 3, 4, 5 as follows:

Table 3 Rating scale for *Fusarium fujikuroi* infected plants, disease development on the leaves and neck (Alberto 2014)

Scale	Description
0	no symptoms
1	slight twisting from the neck
3	slight twisting from the neck with yellow green discolouration of the leaves
5	twisting of the leaves with slight neck elongation
7	twisting of the leaves with severe neck elongation
9	elongated neck with the leaves down to soil surface

Table 4 Rating scale for *Colletotrichum gloeosporioides* infected plants, disease development on the leaves scored (Alberto 2014)

Scale	Description
0	no symptoms
1	1-5 small, whitish water soaked lesions
3	1-2 big, whitish water soaked lesions
5	1-2 prominent lesions with orange fungal growth at the middle of the lesions
7	1 significant lesion with orange acervuli arranged in concentric rings at the centre of the lesions, leaf turn yellowish green
9	1 significant lesion with black acervuli arranged in concentric rings at the middle of the lesions, leaf turn yellow

Table 5 Rating scale for *Fusarium fujikuroi* +*Colletotrichum gloeosporioides* infected plants, disease development on the leaves and neck (Alberto 2014)

Scale	Description
0	no symptoms
1	1-5 small, whitish water soaked lesions with slight twisting from the neck
3	1-2 big whitish water soaked lesions with slight twisting from the neck, leaves turning yellow green
5	1-2 significant lesions with orange fungal growth at the middle of the lesions, twisting of the leaves with slight neck elongation
7	1 significant lesions with orange acervuli arranged in concentric rings at the centre of the lesion, twisted of leaves with severe neck elongation leaf turn green
9	1 significant lesion with black acervuli arranged in concentric rings at the middle of the lesions, leaf turn yellow, elongated neck with leaves

The same protocol was followed to test for the preventive effects of the fungicide treatments and paclobutrazol except that the fungicide treatments (three consecutive applications at 7 days interval) were applied ahead prior to inoculation. A week after inoculation, three more applications at 7 days interval were carried out.

Gathered Data

A week after inoculation, the following parameters were taken and observed for the length of neck and disease development.

$$\textbf{Disease Incidence (\%)} = \frac{\text{number of plants infected}}{\text{Total number of plants}} \times 100$$

$$\textbf{Disease Severity (\%)} = \frac{n(1)+n(3)+n(5)+n(7)+n(9)}{N \times 9} \times 100$$

Where:
n = no. of infected leaves classified by grade
N = total number of leaves/pot examined

Length of the neck – this was taken by measuring the length of the neck, from the base of the leaves of each individual onion plants using a ruler (cm).

Yield (g) – this was taken using a weighing scale.

Results & Discussion

Pathogenicity Test

Disease incidence (%)

Colletotrichum gloeosporioides inoculated plants showed symptoms in onion at 72 hours after inoculation while *Fusarium fujikuroi* inoculated plants showed symptoms at 120 hours, thereafter. The onion plants inoculated with the combination of *Fusarium fujikuroi* and *Colletotrichum gloeosporioides* shows its symptoms at 72 hours after inoculation. The symptoms of plants inoculated with *Fusarium fujikuroi* were observed as yellow green discoloration of the leaves with slight twisting from the neck of onion plants. At the same time, the symptoms of onion plants inoculated with *Colletotrichum gloeosporioides* started as water soaked lesion until it further developed into an orange masses arranged in concentric rings and later turned into black acervulus as it matures. On the other hand, the combination of *Fusarium fujikuroi* and *Colletotrichum gloeosporioides* showed slight twisting from the neck of onions with water soaked lesion that further developed into orange to black masses. Other affected leaves showed dieback and eventually die (Fig. 1). The inoculated isolates were found to be highly pathogenic to onion plants (100% disease incidence) as it shows the typical symptoms occurring in the field (Table 6).

Fig. 1 – (a) Twisting symptoms in onion plants inoculated with *Fusarium fujikuroi* (b) anthracnose symptoms (*Colletotrichum gloeosporioides*) (c) twisting/anthracnose symptoms in onion inoculated with both pathogens.

Table 6 Disease incidence (%) in onion plants infected with *Fusarium fujikuroi* and *Colletotrichum gloeosporioides* 10 days after inoculation

Cultivar	Pathogen*			Control
	F.f	C.g	F.f+C.g	
Yellow Granex	100[a]	100[a]	100[a]	0.00

*F.f = *Fusarium fujikuroi*, C.g = *Colletotrichum gloeosporioides*, F.f + C.g = *Fusarium fujikuroi* + *Colletotrichum gloeosporioides*

Disease severity (%)

At 10 days after inoculation, the disease severity had reached as high as 50.62% in onion plants inoculated with *Fusarium fujikuroi*. Onion plants inoculated with *Colletotrichum gloeosporioides* and *Fusarium fujikuroi* reached disease severity of 70.29% and 80.39%, respectively. Control plants inoculated with sterile distilled water remained unaffected (Table 7).

Table 7 Disease severity (%) in onion plants infected with *Fusarium fujikuroi* and *Colletotrichum gloeosporioides*, 10 days after inoculation

Cultivar	Pathogen*			Control
	F.f	C.g	F.f + C.g	
Yellow Granex	50.62[b]	70.29[a]	80.39[a]	0.00

*F.f = *Fusarium fujikuroi*, C.g = *Colletotrichum gloeosporioides*, F.f + C.g = *Fusarium fujikuroi* + *Colletotrichum gloeosporioides*

Results of pathogenicity tests showed that *Fusarium fujikuroi* and *Colletotrichum gloeosporioides* caused symptoms of deterioration in onion plants. Both pathogens were also proven to be the causal agents of anthracnose and twister syndromes in onions. Also, it shows that the two pathogens were highly virulent to cause disease in onions that requires sensitivity test to fungicides. To satisfy the Koch's Postulates, the two pathogens (*Fusarium fujikuroi* and *Colletotrichum gloeosporioides*) were re-isolated from the previously inoculated onion plants and re-checked its morphology if its characteristics were identical from the characteristics of the original isolates (Fig. 2).

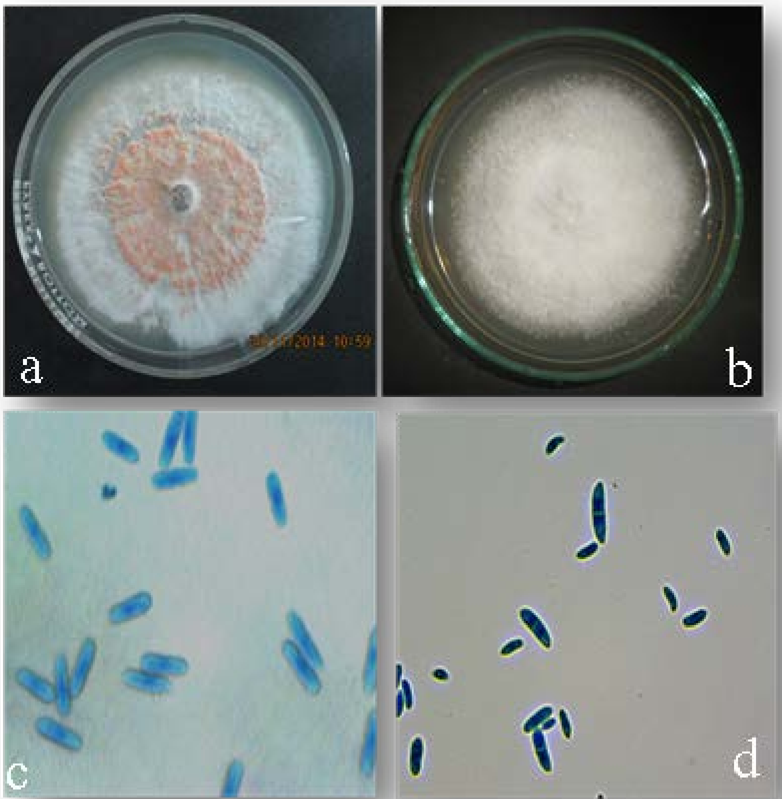


Fig. 2 – Colony morphology of fourteen day old cultures of (a) *Colletotrichum gloeosporioides* (b) *Fusarium fujikuroi*, conidial morphology of (c) *Colletotrichum gloeosporioides* (100x) and (d) *Fusarium fujikuroi* (100x)

Pot Experiment

Disease symptoms

After satisfying the Koch's postulates, the pot experiment was conducted. Disease observation, assessment and evaluation were carried out after inoculation whereby, the symptoms that developed in onions in both components (curative and protective) were used to facilitate data collection. The symptoms in onion plants inoculated with *Colletotrichum gloeosporioides* were whitish lesions and sunken spot with orange dot-like at the center that further developed into orange fungal masses/acervuli arranged in concentric rings (Fig. 3). On the other hand, symptoms inoculated by *Fusarium fujikuroi* was slight twisting leading to the formation of yellow-green discoloration of the leaves, elongation of the neck until the onion leaves were drooping down into the surface (Fig. 4).

Fig. 3 – Common symptoms in onion plants caused by *Colletotrichum gloeosporioides*. a whitish lesion with orange dot-like masses at center. b orange masses arranged in concentric rings. c typical anthracnose symptom.

Fig. 4 – Common symptoms in onion plants caused by *Fusarium fujikuroi*. a slight twisting. b elongated neck. c severe neck elongation with leaves drooping down to the surface.

Disease incidence (%) of Anthracnose-Twister

At six weeks after inoculation of *Fusarium fujikuroi*, the disease incidence in onion plants under protective spray application varies among different chemicals applied. Onion plants treated with Paclobutrazol was free of the disease, while those onion plants treated with Captan, Carbendazim, Carbendazim+Paclobutrazol and Auxin were all infected. On the average, it shows that the onion plants treated with Paclobutrazol has the least incidence of disease along with the onion plants treated with Benomyl, DP, Captan+Paclobutrazol, Benomyl+Paclobutrazol and DP+Paclo, as compared to the Control (+), Captan, Carbendazim, Carbendazim+Paclobutrazol and Auxin treated plants which has the highest occurrence of disease. The disease incidence in onion plants spray inoculated with *Colletotrichum gloeosporioides* alone treated with Captan and Carbendazim+Paclobutrazol was high (85%), in contrast to the onion plants applied with the combination of Captan+Paclo, DP+Paclo and DP (100%) under protective spray application. Taken as a whole, however, onion plants treated with Captan, Carbendazim+Paclo, Benomyl and Carbendazim have lower disease incidence. In contrast, those onion plants treated with Captan+Paclo and DP got the highest percentage of disease incidence. The incidence of disease in onion plants inoculated with the two pathogens (*Fusarium fujikuroi* +*Colletotrichum gloeosporioides*) was found to be low in onion plants treated with Paclobutrazol (54.51%) and highest in Auxin treated plants (100%) under protective spray application. Among the different treatments, those treated with Paclobutrazol had the lowest disease incidence, followed by Captan and Benomyl+Paclo (Fig. 5).

On the other hand, results under curative set-up demonstrated how ineffective the chemicals are when applied as a curative spray, as evidenced by high disease incidence in all of the treatments. Based from the results, the establishment of disease before the application of chemicals can cause substantial damage in the onion plants (Fig. 6).

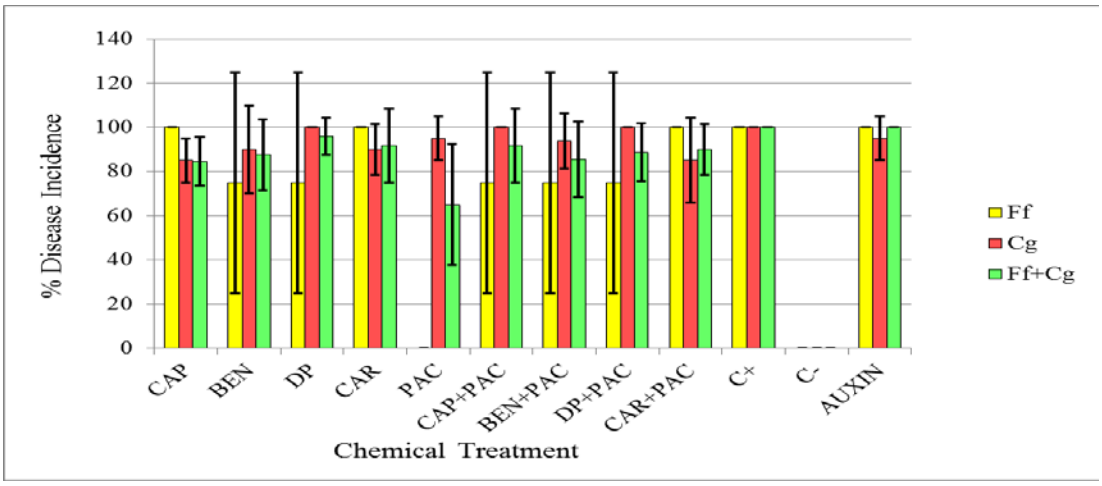


Fig. 5 – Disease incidence (%) of onion plants inoculated with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* (Ff) + *Colletotrichum gloeosporioides* (Cg) under protective spray application.

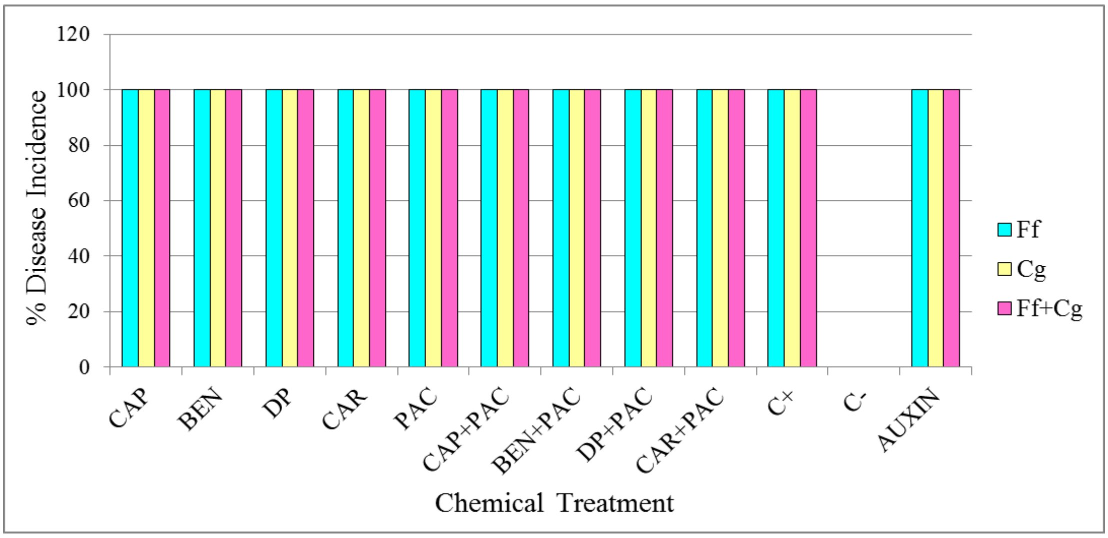


Fig. 6 – Disease incidence (%) of onion plants inoculated with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* (Ff) + *Colletotrichum gloeosporioides* (Cg) under curative spray application.

Disease Severity (%) of Anthracnose-Twister

The disease severity in onion plants inoculated with *Fusarium fujikuroi*, *Colletotrichum gloeosporioides* and its combination under protective spray application was found to be low (Fig. 7). Taken as a whole, the outcome showed that the onion plants treated with Paclobutrazol

(Fig. 8) has no symptoms at all as well as those plants treated with the combination of Carbendazim+Paclobutrazol (2.78%). On the contrary, the onion plants treated with Auxin and Carbendazim showed the most severe symptoms.

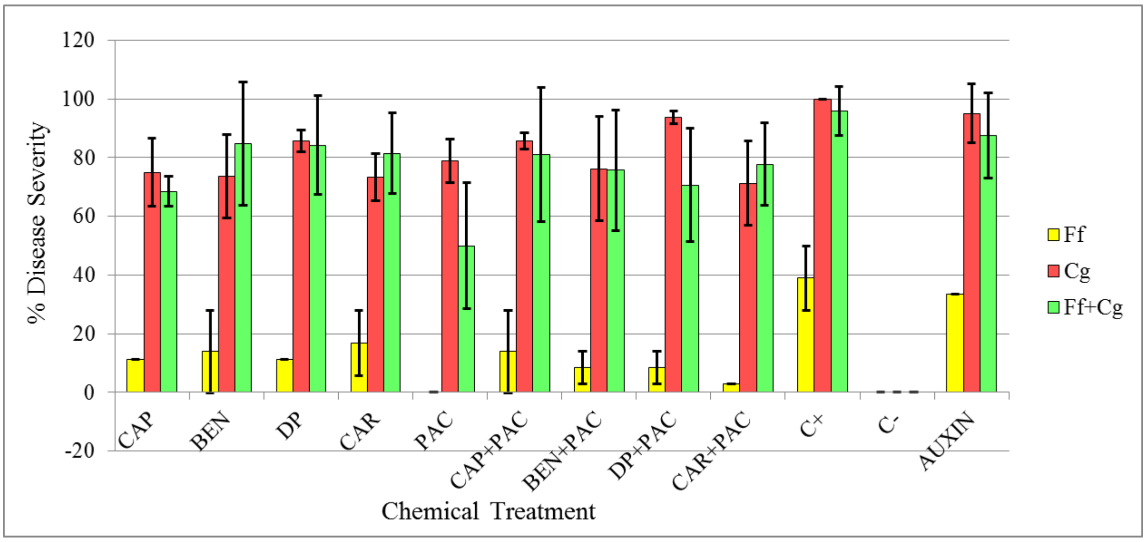


Fig. 7 – Disease severity (%) of onion plants inoculated with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* (Ff) + *Colletotrichum gloeosporioides* (Cg) under protective spray application.

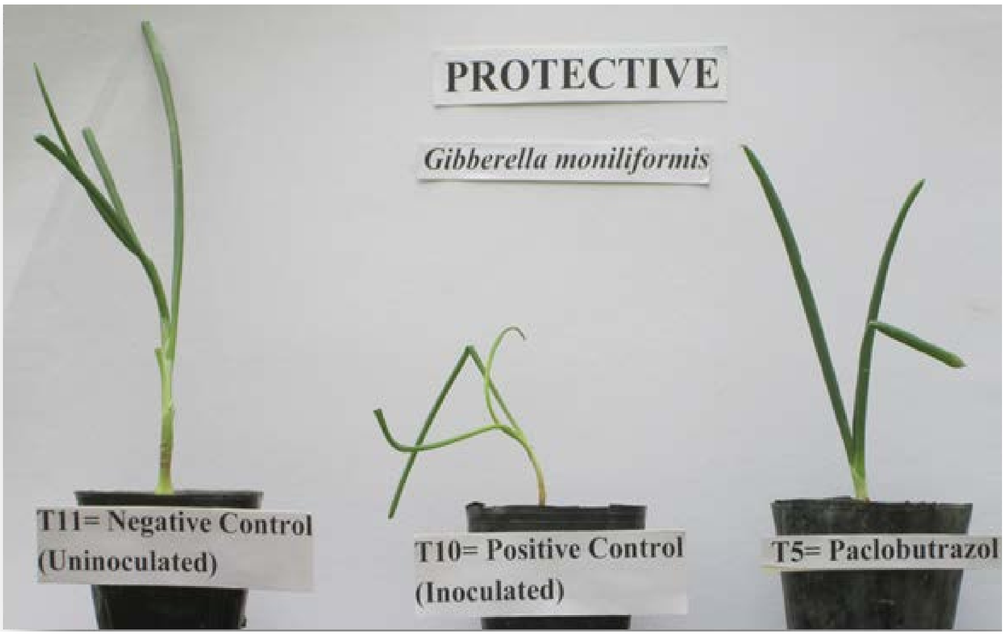


Fig. 8 – *Fusarium fujikuroi* infected onion plants treated with Paclobutrazol under protective spray application.

The lowest percentage of disease severity spray inoculated with *Colletotrichum gloeosporioides* was observed in onion plants treated with Carbendazim+Paclobutrazol (71.30%) (Fig. 9) followed by Carbendazim with 73.33% and Benomyl with 73.61%, respectively. Unlike in onion plants treated with Auxin, the severity reached as high as 95% along with DP+Paclobutrazol with 93.70% that shows the most severe symptoms and the highest percentage of disease severity. On average, the response of onion plants when treated with Carbendazim+Paclobutrazol as protective spray had the least disease severity.

The disease severity of onion plants infected with the combination of the *Colletotrichum gloeosporioides* and *Fusarium fujikuroi* was found to be low in Paclobutrazol (50%) treated plants together with Captan (68.47%) and DP+Paclobutrazol (70.69%). In the contrary, onion plants treated with Auxin (87.50%) and Benomyl (84.72%) were observed to have the highest disease severity. The symptoms of the disease were observed to have the least severity in onion plants treated with Paclobutrazol (Fig.10).

Under curative spray application infected with *Fusarium fujikuroi*, onion plants treated with Auxin (33.33%) have the highest percentage of disease severity followed closely by plants applied with Captan (27.78%). Quite the opposite, the lowest disease severity was observed in onion plants treated with Ben+Paclo (11.11%) together with the DP+Paclo (11.11%) (Figs 11, 12).

The severity of onion plants, under curative spray application inoculated with *Colletotrichum gloeosporioides* was lower in Carbendazim (81.11%) and Carbendazim+Paclobutrazol (86.67%) in contrast to onion plants treated with Auxin, DP and DP+Paclobutrazol with 100% disease severity respectively. Over-all, the least disease severity was observed in onion plants treated with Carbendazim and higher severity was obvious when the different chemicals were applied as curative sprays (Figs 11, 13).

Under curative spray application infected with both pathogens (Ff+Cg), the lowest disease severity was observed in onion plants treated with Benomyl with 73.61% followed by Carbendazim (81.30%) and DP with 81.71%, unlike in onion plants treated with Auxin, wherein, the severity had reached as high as 96.67% along with Captan with 97.04% which shows the most severe symptoms and the highest percentage of disease severity. In general, the least disease severity was showed in onion plants treated with Benomyl while other chemical treatments do not vary that much when applied as curative spray (Figs 11, 14).

Fig. 9 – *Colletotrichum gloeosporioides* infected onion plants treated with Carbendazim+Paclobutrazol under protective spray application.

Disease Progress (% Incidence) of Anthracnose-Twister

On the 14th day after inoculation of *Fusarium fujikuroi*, symptom development continues to progress up to 21th day, thereafter, the incidence of the disease in all treated plants were high ranging from 92.50% -100% under curative spray application. Also, the symptoms also started to progress from day 14 which have persisted until day 42 and reached 77.50% of disease incidence despite weekly chemical application (Fig. 15B). Though a similar trend on disease progression was observed in both spray applications, the difference on the degree of disease incidence was demonstrated.

Fig. 10 – *Colletotrichum gloeosporioides+ Fusarium fujikuroi* infected onion plants treated with Paclobutrazol under protective spray application.

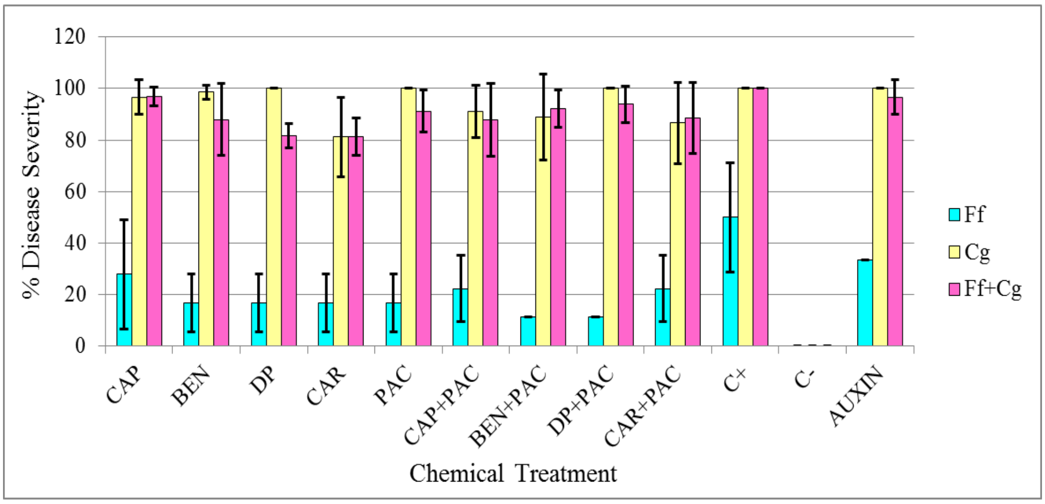


Fig. 11 – Disease severity (%) of onion plants inoculated with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* (Ff) + *Colletotrichum gloeosporioides* (Cg) under curative spray application.

The progress of disease incidence (%) inoculated with *Colletotrichum gloeosporioides* shows a similar trend in onion plants treated with different chemicals both under curative and protective spray application (Fig. 15). Under curative spray application, high incidence of disease was observed on 7th day (62.92%) after inoculation. Furthermore, the incidence of the disease increases from 97%-100%, which were observed from 28th days until 42nd day. Whereas, under protective spray application, the incidence of disease on the 7th day was recorded at 58.67%, as its symptoms continue to develop until 21st day. A higher incidence was observed from 28th day up to the 42nd day with an average of 87.92%-93.38% disease incidence.

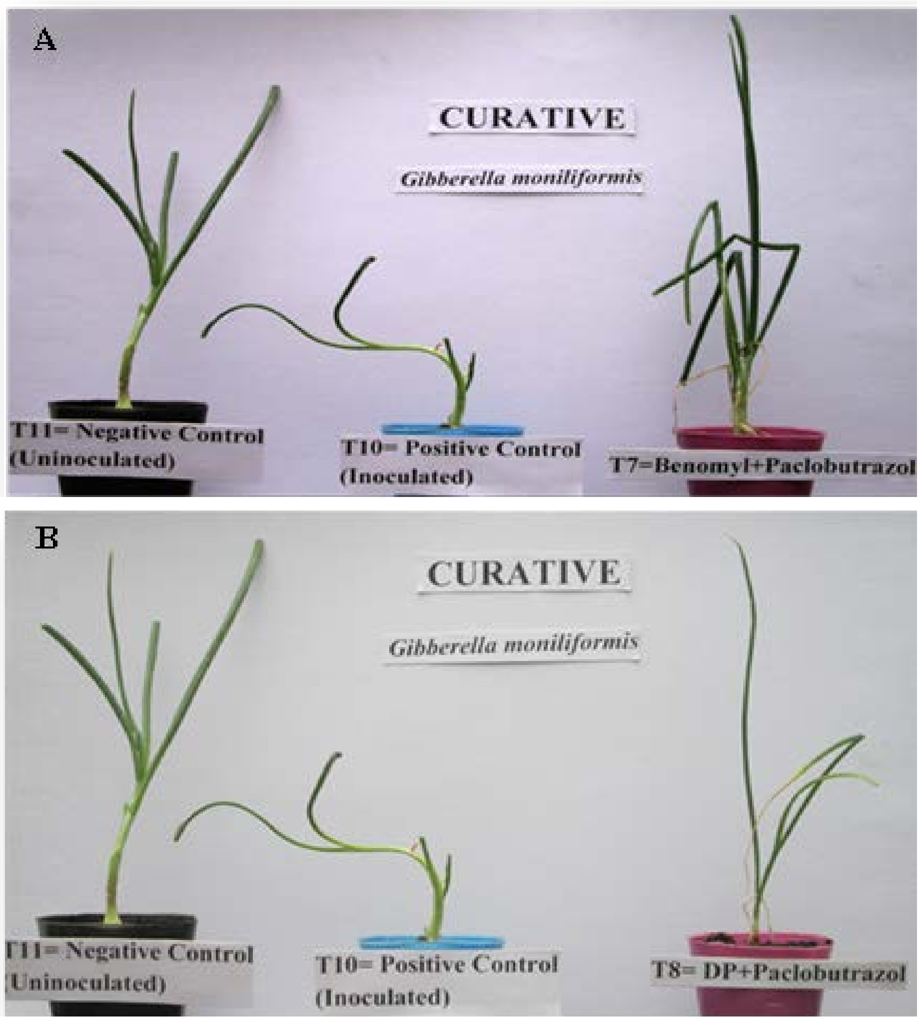


Fig. 12 – *Fusarium fujikuroi* infected onion plants treated with (A) Benomyl+Paclobutrazol and (B) DP+Paclobutrazol under curative spray application.

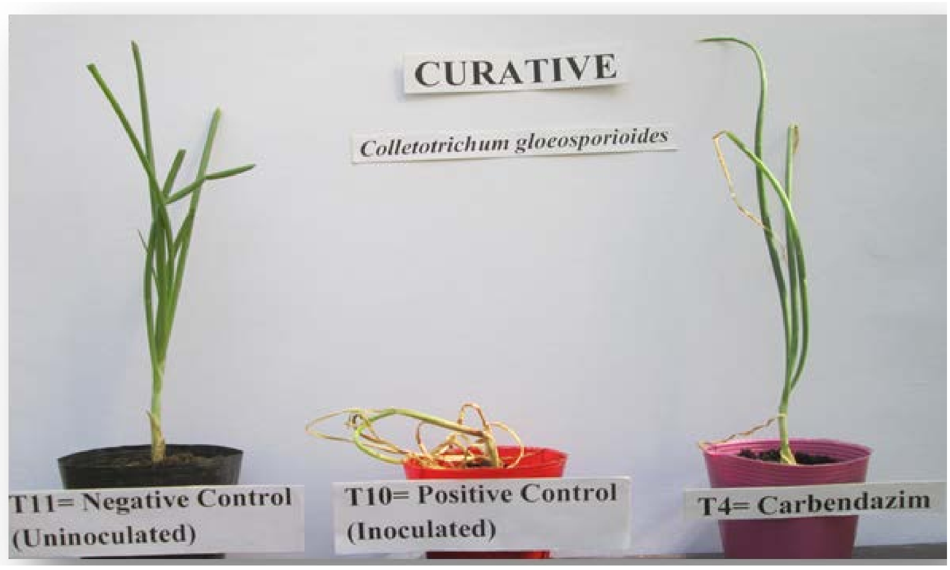


Fig. 13 – *Colletotrichum gloeosporioides* infected onion plants treated with Carbendazim under curative spray application.

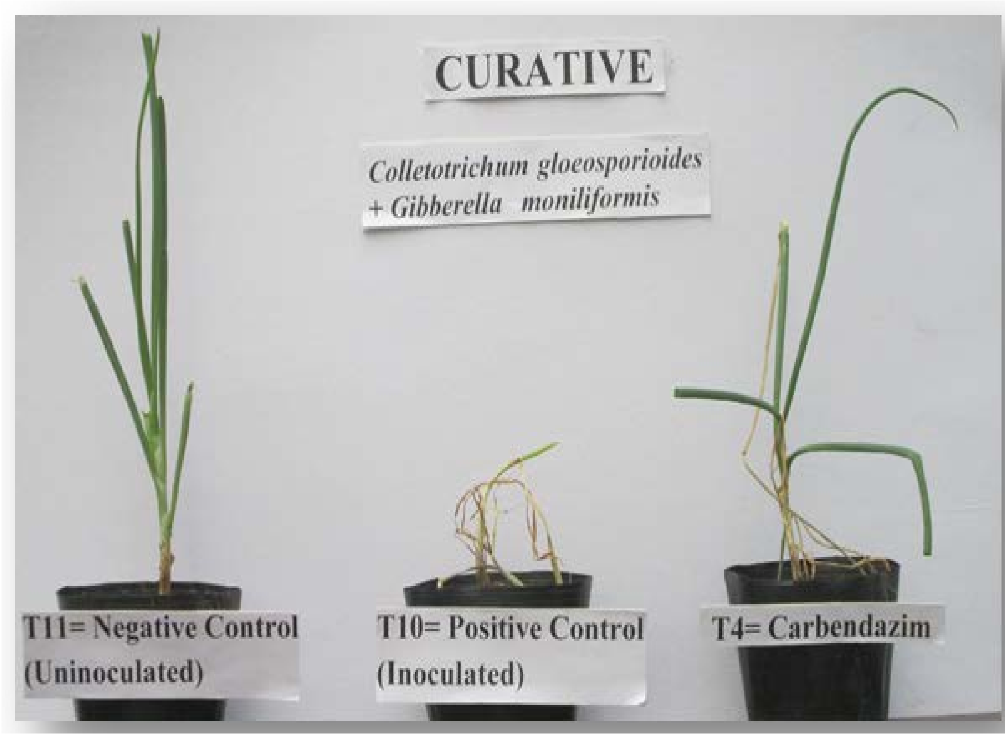


Fig. 14 – *Colletotrichum gloeosporioides+ Fusarium fujikuroi* infected onion plants treated with Carbendazim under curative spray application

Onion plants infected with *F. fujikuroi* + *C. gloeosporioides* under curative spray application continue to develop its symptom from the 7th day up to the 21st day after inoculation Figure 15B.). Subsequently, from 21st day-28th day, disease development seems to slow down from 83.17%-84.58%. Furthermore, as it reached the 42nd day, the highest incidence was attained at 99.38%. Whereas, in protective spray application, the symptoms started to progress from day 7, which persisted until day 42 with 88.04% of disease incidence (Fig. 15A).

Disease Progress (% Severity) of Anthracnose-Twister

On the 7th day after inoculation of *F. fujikuroi*, symptom development continued up to the 21th day, then after that, the disease severity in all treated plants increases from 18.61%-19.44% under curative spray application. While in protective spray application, the symptoms also started to appear on day 7 and developed continuously until day 42 but with only 12.22% disease severity (Fig. 16A).

Similar response of onion plants treated with different chemicals both under curative and protective spray application inoculated with *C. gloeosporioides* was observed. Though severity on plants under protective spray application was obviously lower than those plants under curative spray application (Fig. 16).

Furthermore, the high severity of symptoms continues to progress from 84.99%-94.31%, which were observed on 28th days until 42nd day. While, onion plants under protective spray application, the severity of disease was only 26.04%, on the 7th day, continue to develop up to the 21st day. The disease turned out to be more severe, starting on the 28th day up to the 42nd day.

Under curative spray application infected with *F. fujikuroi* + *C. gloeosporioides*, it shows continues symptom development from 7th day until 21st day after inoculation. As it reached 42nd day, the most severe symptoms were observed (89.82%) under protective spray application. The symptoms started to progress from day 7 which persisted until day 42, where the symptoms became less severe (76.16%) (Fig. 16B).

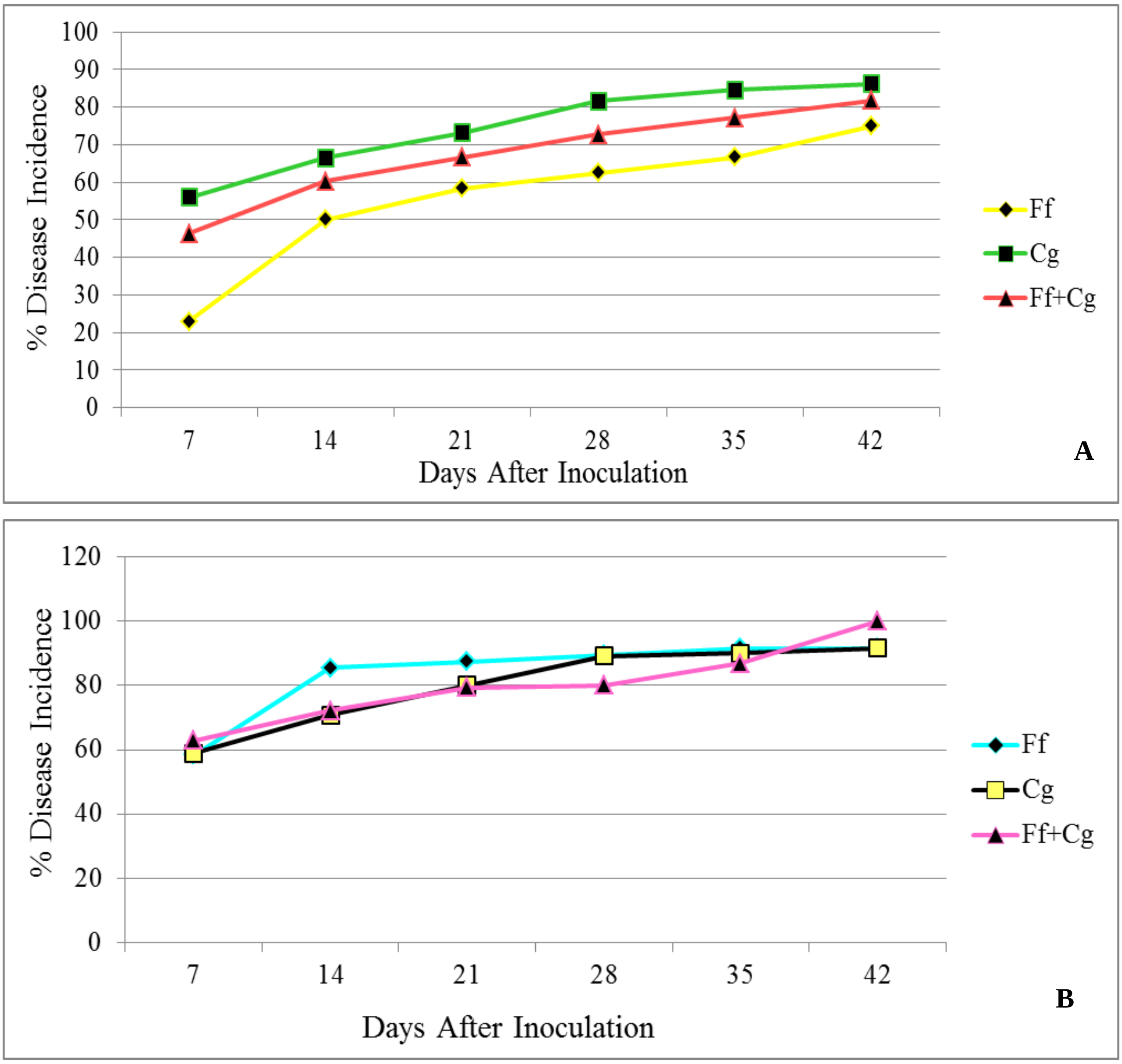
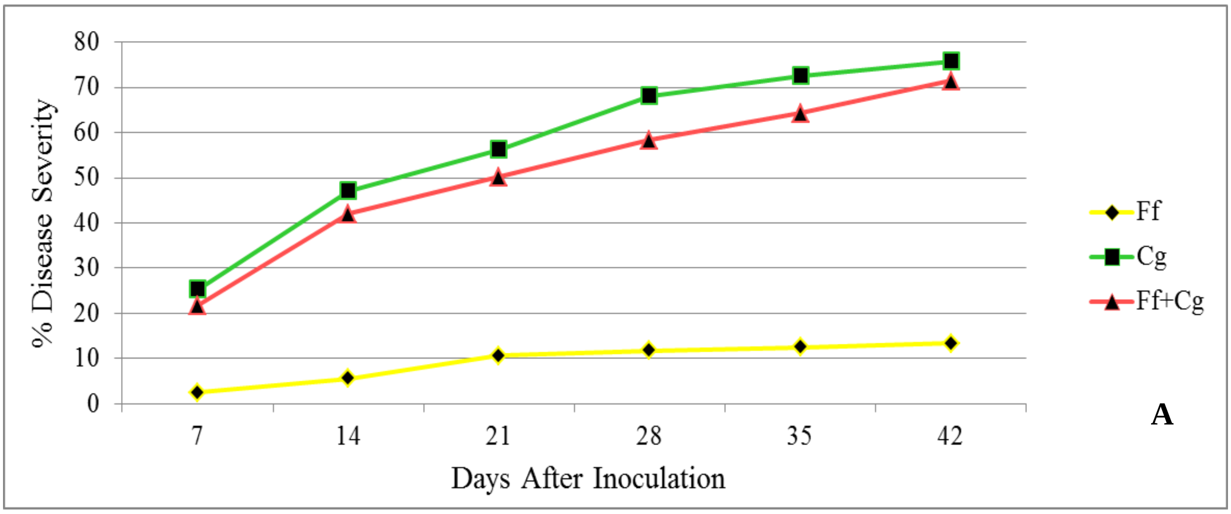


Fig. 15 – Progress of disease incidence in onion plants infected with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* + *Colletotrichum gloeosporioides* (Ff+Cg) under (A) protective and (B) curative spray applications.

Length of Neck (cm)

Under protective spray application, *Fusarium fujikuroi* inoculated onion plants treated with Paclobutrazol was observed to have the shortest length of the neck (1.55 cm). In contrast to Auxin (6.13 cm) where the longest length of neck was observed followed by Captan (6.00 cm) and Carbendazim (6.00 cm). Based on the results, onion plants applied with Paclobutrazol showed the shortest length of neck. According to Davis (1988), plants treated with paclobutrazol typically have shorter internodes and thicker green leaves. On the study of Mactal (2011) in rice, paclobutrazol is effective in shortening the length of the lower internodes and inhibits stem elongation resulting to shorter plants with increased lodging resistance. Onion plants infected with *Colletotrichum gloeosporioides* whereby the chemicals were applied as protective sprays showed that those onion plants treated with Paclobutrazol have the shortest length of neck (2.50 cm) together with Benomyl+Paclobutrazol (3.05 cm). In the contrary, onion plants treated with Auxin (4.75), together with the combination of Captan+Paclobutrazol (4.23 cm) and Carbendazim+Paclobutrazol (4.23 cm) had the longest length of neck. The onion plants under protective spray application and infected with both pathogens (*Fusarium fujikuroi* +*Colletotrichum gloeosporioides*), treated with Paclobutrazol (3.00 cm) and Benomyl+Paclobutrazol (4.25 cm) have the shortest length of neck, while the onion plants treated with Carbendazim and Auxin had the longest neck measuring 7.00 cm and DP with 6.88 cm under (Fig. 17).



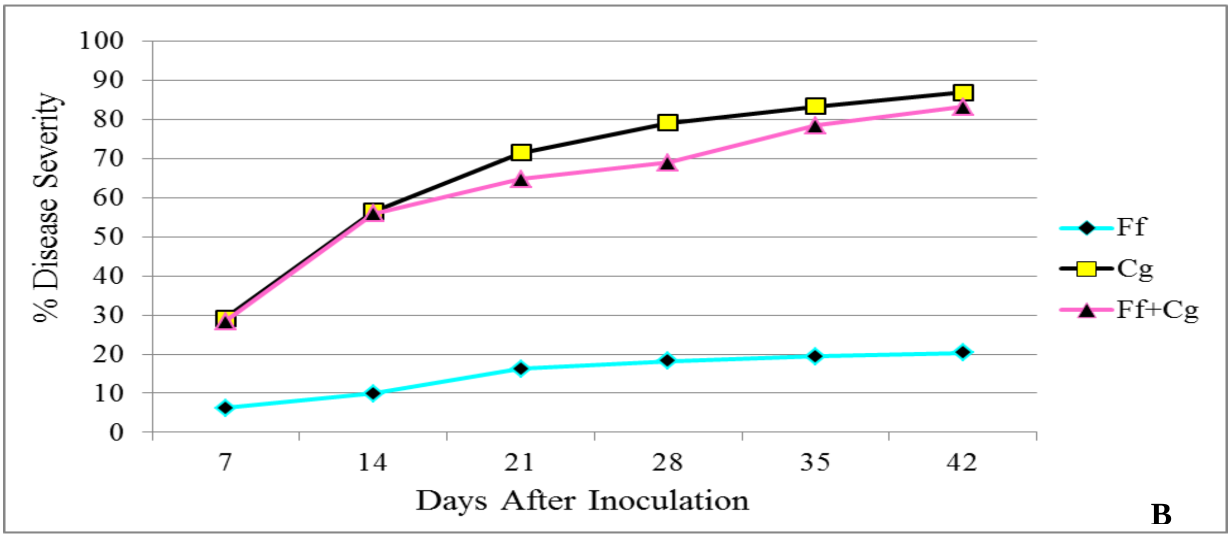


Fig. 16 – Progress of disease incidence in onion plants infected with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* + *Colletotrichum gloeosporioides* (Ff+Cg) under (A) protective and (B) curative spray applications.

Onion plants inoculated *Fusarium fujikuroi* treated with DP+Paclobutrazol (3.75 cm) has the shortest length of neck along with DP (4.50 cm) and Benomyl+Paclobutrazol (4.63 cm). Onion plants applied with Auxin (6.25 cm) together with Carbendazim (6.00 cm) and Captan+Paclobutrazol (6.00 cm) showed the longest length of the neck under curative spray application. Onion plants infected with *Colletotrichum gloeosporioides* and treated with Paclobutrazol have the shortest length of neck (3.63 cm) followed by Benomyl+Paclobutrazol (3.90 cm) as compared to the onion plants treated with DP (5.38 cm), Carbendazim (5.38 cm) together with Captan (5.25 cm) which has the longest length of neck under curative spray application . On the other hand, onion plants inoculated with *Fusarium fujikuroi* and *Colletotrichum gloeosporioides*, shows that onions applied with DP (7.05 cm), Benomyl+Paclobutrazol (7.13 cm) and Captan+Paclobutrazol (7.38 cm) were observed to have the shortest length of neck, unlike the onion plants treated with DP+Paclo (8.50 cm), Carbendazim (8.00 cm) and Captan (8.00 cm) where the necks were much longer (Fig. 18).

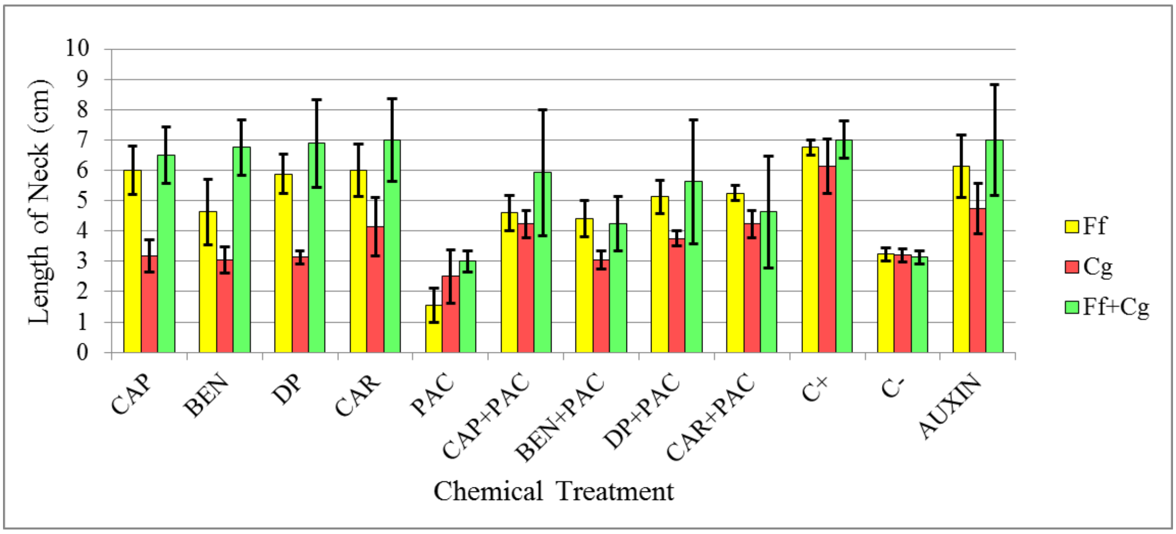


Fig. 17 – Length of neck (cm) in onion plants infected with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* (Ff) + *Colletotrichum gloeosporioides* (Ff+Cg) under protective spray application

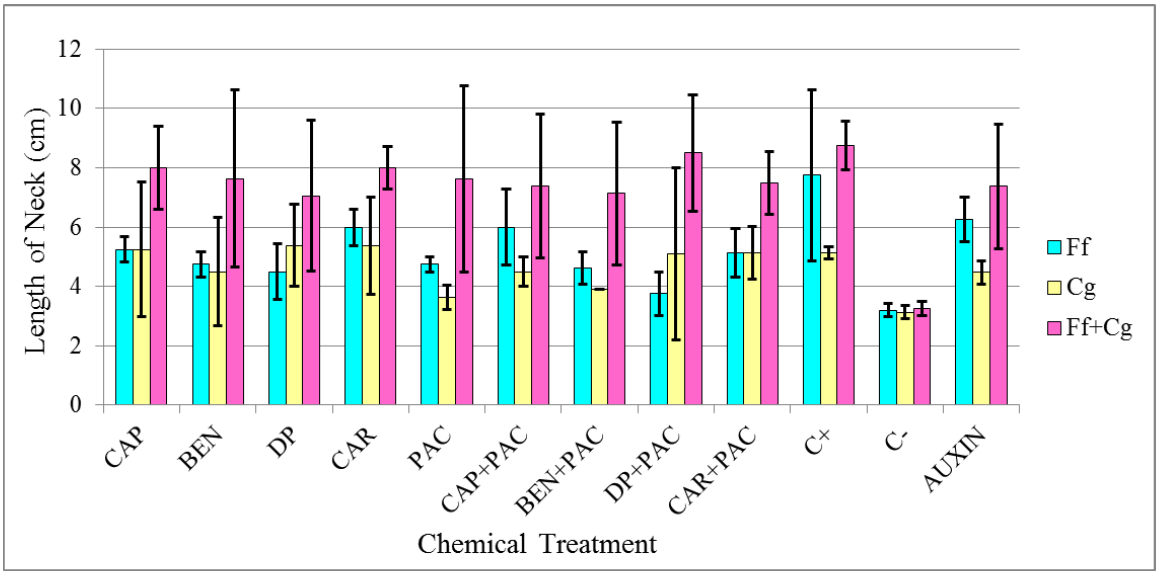


Fig. 18 – Length of neck (cm) in onion plants infected with *Fusarium fujikuroi* (Ff), *Colletotrichum gloeosporioides* (Cg) and *Fusarium fujikuroi* (Ff) + *Colletotrichum gloeosporioides* (Ff+Cg) under curative spray application

Yield (g)

The yield response of onion plants under protective spray application showed Captan treated plants (20.81g) had the highest yield, followed by Carbendazim+Paclobutrazol (18.46g) and Carbendazim (18.06g). On the other hand, onion plants applied with Paclobutrazol (9.51g), Auxin (9.34g) and DP+Paclobutrazol (10.59g) gave the lowest yield. Based from the results, performance of Paclobutrazol towers over the performance of other treatments in all the parameters tested except in yield where it has the lowest. The bulbs were small, circular but light. In strawberries, Malcolm & Carol (1986) found that paclobutrazol had little effect on total yield or proportion of strawberry fruit per size category. Paclobutrazol treated plants were smaller in size than control and their runner production was inhibited. Ramina & Tonutti (1985) also reported that the chemical without affecting the yield significantly reduced vegetative growth. Assem (1986) found that use of paclobutrazol in berries clearly showed an increase in chlorophyll content, berry set, number of berries per cluster and cluster weight. However, a reduction in berry size was noticed depending on the concentration and time of application. Under curative spray application, onion plants treated with Captan were observed to have the highest yield with 13.65 g followed by Captan+Paclobutrazol (13.29g) and Benomyl+Paclobutrazol (12.62 g). While the lowest yield was observed in onion plants treated with Auxin (6.18 g) and DP (8.22 g) (Fig. 19).

The over-all yield response of onion plants showed an immense variation as chemicals were applied as protective and curative. Under protective spray application, treated onion plants were observed to have a higher yield as compared on onion plants treated with chemicals which were applied as curative sprays (Fig. 20).

The over-all yield response in treated onion plants showed that protective spray application was better as compared to curative spray application. Considering that the pathogens were already established inside the plant, thus making the pathogens out of reach by the chemicals, so much so that most of the chemicals tested were protectant in nature. On the other hand, the onion plants under protective spray application showed lower disease incidence and severity due to the protective effect given by the chemical treatments as it provides protective cover to the onion plants by killing the spores prior to germination.

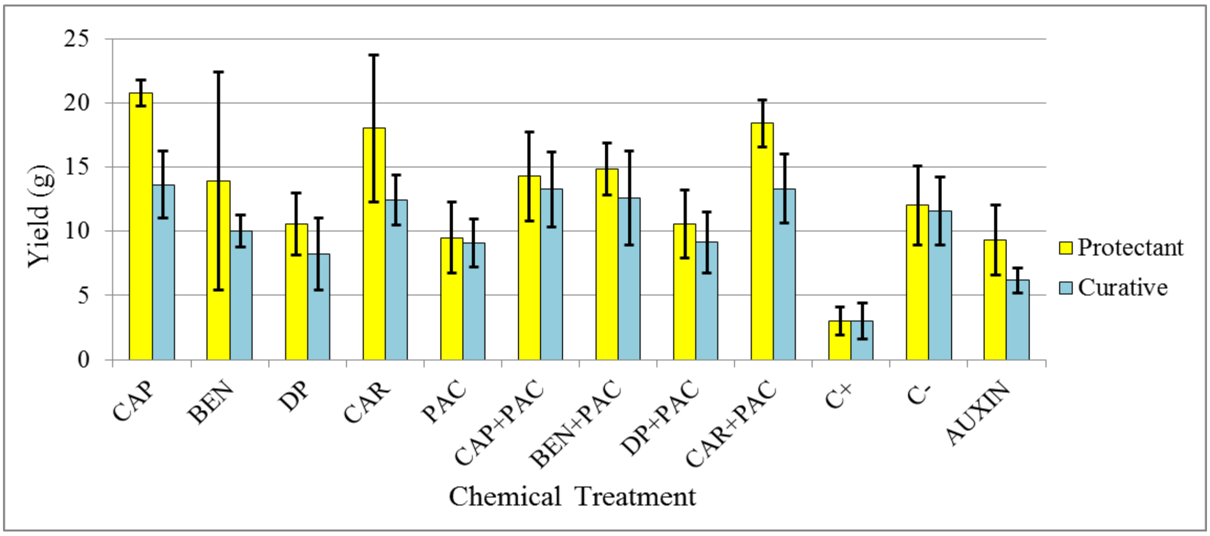


Fig. 19 – Yield response of onion plants under protective and curative spray application.

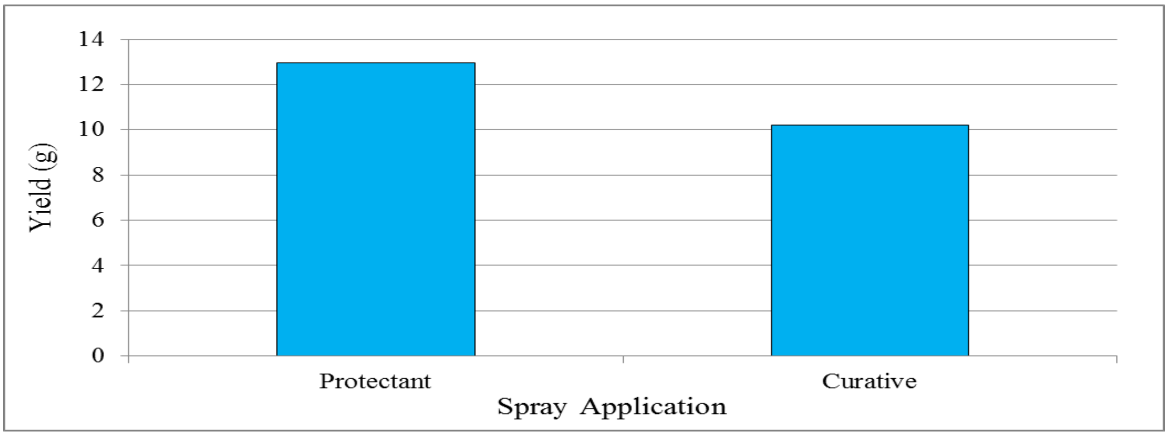


Fig. 20 – Yield response of treated onion plants under curative and protective spray application

Summary, Conclusion and Recommendation

The different commercial fungicides, namely; Captan (Captan 50WP), Benomyl (Venom), DP (Armure 300EC) and Carbendazim (Bavistrin) were evaluated against anthracnose-twister disease. The paclobutrazol was also tested with regards to the suppression of rapid production of gibberellic acid that causes the development of twister disease. The performance of paclobutrazol and the test fungicides in suppressing anthracnose-twister was compared using curative and protective spray applications. It was reported before that to lessen or protect the onion plants from infection, there was in need to apply protectant fungicides {Captan, Mancozeb (Dithane) or Benomyl (Benlate)} one week after transplanting or one week after emergence for direct seeded onion. Repeat application at 7–14 days interval depending on the severity of the disease. Or use systemic foliar fungicides {e.g., Armure (Mancozeb, Difenoconazole/Propiconazole), Score (Difeconazole)} two weeks after transplanting. Repeat application at 7–14 days interval depending on the severity of the disease. Use sticker and spreader when spraying fungicides. (CLARRDEC 2007). This disease can be treated with the protectant fungicides mancozeb (e.g. Dithane or Manzate) Or chlorothalanil (Bravo, Equus, Echo, etc.). The strobilurin fungicides Quadris and Cabrio are also effective but must be used in a rotation with a protectant to prevent the development of fungicide resistance (Miller 2013). On the other hand, paclobutrazol is a potent regulator of gibberellin biosynthesis and inhibits the oxidation of kaurene to kaurenoic acid. Specifically, it interacts with kaurene oxidase, a cytochrome P-450 oxidase, and inhibits the microsomal oxidation of kaurene, kaurenal, and kaurenol (Hedden & Graebe 1985). Reduced levels of gibberellins lead to a decrease in cell division and elongation at the apical meristem of the shoot but has little effect on the production of leaves or root growth (Gianfagna 1995).

Six weeks after inoculation, high incidence of disease was very evident in all onion plants treated with different chemicals under curative spray application. Moreover, DP and Auxin treated plants manifested the most severe symptoms which were similarly recorded on both spray applications.

On the other hand, onion plants applied with Captan and Paclobutrazol showed the least incidence of disease under protective spray application. The performance of the fungicide Carbendazim and Paclobutrazol evidently showed the least severity of the disease.

The shortest length of the neck (cm) was observed in all onion plants treated with Paclobutrazol and also shows no symptoms of twisting under protective spray application. Whereas, the longest neck was recorded in onion plants treated with Auxin and Carbendazim.

The onion plants treated with Captan and Carbendazim+Paclobutrazol had the highest yield, on the contrary, the response of onion plants applied with Auxin and DP showed the lowest yield.

Protective spray application obviously showed lower disease incidence and severity, shorter neck and higher yield as compared to curative spray application.

Since, this study was conducted under pot experiment; field trials must be carried out for further validation of the results and to get reliable information on the effectiveness of the chemicals. Further study and experiments must be conducted with the chemicals Paclobutrazol and Auxin as regards to their ability in suppressing twister disease.

Acknowledgement

The author would like to acknowledge the Institute of Climate Change and Environmental Management (ICCEM) located at Central Luzon State University and Syngenta Phils Inc. for supporting and funding this experiment.

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