

The Effects of Fungicides Alone and in Conjunction with Chitin on the Control of Some Fungal Pathogens Associated with Chilli Seeds

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Abstract: Various species of pathogenic fungi were isolated from chilli seeds using of standard blotter and agar plate methods. Among the isolates *Alternaria alternata*, *A. solani*, *Aspergillus flavus*, *A. niger*, *Cercospora capsici*, *Colletotrichum capsici*, *Drechslera hawaiiensis*, *Fusarium oxysporum*, *F. solani*, *Leveillula taurica*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Phytophthora capsici* and *Pythium* spp., were common. Seed treatment with four fungicides alone and in conjunction with chitin viz., Aliette (80% WP (Wettable Powder) Fosetyl aluminium), Antracol (70% Propineb), Score (250 EC (Emulsifiable concentrate) Defenaconazole) and Cabrio Top (60% WDG (Water Dispersible Granules) Pyraclostrobin: Metiram) were used at the concentration of 0.10%, 0.20% and 0.30%. Out of these four fungicides; Aliette at 0.20% and 0.30% alone and together with chitin inhibited the growth of all the tested fungi and proved to be more efficacious than other fungicides.

Key words: Chilli • Fungi • Chitin and fungicide

INTRODUCTION

Chilli is among the world's most popular vegetable crop belonging to the family Solanaceae [1]. Several abiotic and biotic stresses often affect the productivity of chilli crop worldwide. In addition to fungal, bacterial, nematodes and viral diseases are also responsible for significant production constraints affecting both yield and quality and are often difficult to control [2]. The pathogens attack roots, stems, leaves and fruits of the pepper plant and cause 70% to 100% yield loss [3]. In Pakistan, several plant parasitic pathogens which cause root rot and fungi including *Fusarium* spp. produce wilt, root rot while powdery mildew is caused by the fungus *Leveillula taurica*, damping off and wilting of seedlings caused by *Pythium aphanidermatum* and root rot and wilting of chilli plant caused by *Macrophomina phaseolina* [4-8]. Chilli is susceptible to several diseases including root and collar

rot produced by *Phytophthora capsici* [5, 9-13], anthracnose or die-back and fruit rot caused by *Collectotrichum* spp., [14-16]. *Rhizoctonia* root rot caused by *R. solani* generally affects seedlings, but *R. solani* can also infect mature plants and induce root rot, which leads to wilting and death of chili plants. To date, there are no commercially acceptable chili cultivars that are resistant to *R. solani* [17]. In view of unavailability of disease resistant varieties the farmer has to resort to the use of fungicides for the control of diseases of chilli caused by fungal pathogens. Chitin or chitosan are naturally occurring compounds that have great potential in agriculture with regard to managing fungal diseases [18-20]. As far as we know, no attempt has been made to use fungicide and chitin together for the control of fungal pathogens.

Chitin, a β -(1,4)-linked homopolymer of N-acetyl-D-glucosamine (GlcNAc) and β -(1,3/1,6)-D-glucans are known to be ubiquitous components of walls of higher

fungi [21-24]. Chitin in fungi can be detected by chemical methods [25-27] and by cytochemical techniques using either chitinase-gold complexes [28] or the wheatgerm agglutinin (WGA) which binds strongly to GlcNAc residues [29, 30]. The use of WGA in conjunction with colloidal gold has demonstrated the localization of GlcNAc within cell walls of different plant pathogenic fungi such as anthracnose fungus [31], vascular disease-causing fungi [21] and scleroderma canker agents [32].

Chitin was reported as resistance inducer against soil-borne diseases [33, 34, 35]. Addition of small quantities of chitin to soil resulted in a marked reduction in root rot diseases of some plants [34-36]. Furthermore, chitosan, a similar compound, is a safe material which has antifungal activity against many plant pathogens [37, 38]. Chitosan, a non-toxic compound was reported to induce resistance against soil-borne fungi [39-42].

In this paper we attempt to evaluate the efficacy of four different fungicides either alone or in combination with chitin.

MATERIALS AND METHODS

For the isolation of various fungi following methods were used.

Standard Blotter paper Method: Using standard blotter paper method [43], 200-400 seeds of each samples were used and 10-20 seed placed on three well soaked filter papers in 9cm diameter Petri dishes were incubated at $25\pm 1^\circ\text{C}$ under 12 h alternating system of ADL (Artificial Daylight supplied by cool white fluorescent tubes) and examined under stereomicroscope for the presence of fungi, 5-6 days after incubation. Fungi encountered on blotters were identified up to the species level. In comparable set, seeds were disinfected with 1% sodium hypochlorite for 3 minutes before placing them on blotter papers [44].

Agar Plate Method: For agar plate or Ulster [45] method, seeds were placed on PDA in 9cm diameter Petri plates and 10-20 seeds per plate per treatment were used for the experiment. In a comparable set the seeds were surface sterilized with 1% sodium hypochlorite for 3 minutes, washed in sterilized distilled water before plating them on PDA. After 5-6 days incubation the fungi were identified [44].

Identification of Isolates: Isolated fungi were identified using standard references [46-52].

RESULTS AND DISCUSSION

Evaluation of four fungicides and fungicide together with chitin viz., Aliette (80% WP), Antracol (70%), Score (70% EC) and Cabrio Top (60% WDG) were carried out at 0.10%, 0.2% and 0.3% on *Alternaria alternata*, *A. solani*, *Aspergillus flavus*, *A. niger*, *Cercospora capsici*, *Colletotrichum capsici*, *Phytophthora capsici*, *Drechslera hawaiiensis*, *Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Pythium* spp., *Phytophthora capsici* and *Rhizoctonia solani* of chilli diseases. For this purpose, Standard blotter and Agar plate methods were used.

In Standard blotter method, 200-400 seeds of each samples were used and 10-20 seed placed on three well soaked filter papers in 9cm diameter Petri plates were incubated at $25^\circ\text{C} (\pm 1^\circ\text{C})$ under 12 h alternating system of ADL (Artificial Daylight supplied by cool white fluorescent tubes) and examined under stereomicroscope for the presence of fungi, 5-6 days after incubation. In comparable set, seeds were disinfected with 0.10%, 0.20% and 0.30% concentrations of fungicides alone and as well as in conjunction with chitin by the same concentrations.

Results of all fungicides alone in Standard blotter paper method showed the growth suppression of all the tested fungi. Out of all 4 fungicides, Aliette (80% WP) at 0.30% concentration most effectively controlled the fungi. Whereas, 0.20% dose also reduced the growth of the fungi tested to a considerable extent. Antracol (70%) at 0.30% also showed suppression of all the isolated fungi as compared to the same doses of Cabrio Top (60% WDG) and Score (250 EC) (Table 1). The infection percentage and treatment of fungicides only on the growth of isolated fungi by blotter paper method are shown in Figure 1

The results of fungicide application in conjunction with chitin using blotter paper method showed the suppression of growth of all the tested fungi compared to fungicides used alone and out of various test fungicides together with chitin Antracol (70%) at 0.30% concentration controlled the fungi most. Whereas, 0.20% dose also reduced the growth of all the fungi to some extent. Whereas, Aliette (80% WP) at 0.30% also controlled all the isolated fungi as compared to the same doses of Score (250 EC) and Cabrio Top (60% WDG) (Table 4). The infection percentages and treatment of fungicides with chitin on the growth of isolated fungi by blotter paper method are shown in Figure 2.

Table 1: Mean and Standard error of the occurrence of fungi in fungicide treatments alone by Standard blotter paper method

Treatments	0%	0.10%	0.20%	0.30%
Aliette 80% WP	41.84±4.39	1.28±0.54	0.6±0.40	0.3±0.24
Antracol 70%	41.84±4.39	2.76±1.40	1.53±0.94	0.49±0.35
Score 250 EC	41.84±4.39	2.86±0.67	1.72±0.45	0.72±0.23
Cabrio Top 60% WDG	41.84±4.39	3.72±1.48	1.77±0.74	0.54±0.25

Table 2: Percentage occurrence of fungi after treatment with different fungicides alone and together with chitin by Standard Blotter paper method

Name of Fungi	Control	Aliette 80% WP			Antracol 70%			Score 250 EC			Cabrio Top 60% WDG			Aliette 80% WP+Chitin			Antracol 70% +Chitin			Score 250 EC+Chitin			Cabrio Top 60% WDG+Chitin		
		0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
<i>Alternaria alternata</i>	34	0.5	0	0	4.2	1.6	0	2.3	1.2	0.8	5.3	2.6	1.1	0.2	0	0	1.7	0.1	0	1.3	0.7	0	3.3	2.1	0.7
<i>A. solani</i>	33.5	0.2	0	0	1.2	0	0	1.9	1.6	0.3	1.4	0.5	0	0	0	0	0.5	0	0	1.1	0.2	0	1.1	0.1	0
<i>Aspergillus flavus</i>	68.4	3.2	1.5	0.2	7.7	5.6	2.2	7.5	4.6	2.1	9.6	4.9	2.5	1.3	0.5	0	2.3	1.3	0.1	5.3	2.7	1.6	8.5	3.2	2.1
<i>A. niger</i>	65	6.5	5.1	3.2	17.5	12	4.2	8.2	5.1	2.6	19.5	9.5	2.3	4.3	2.1	1.1	11.7	6.4	0.7	6.7	2.3	1.3	17.5	8.1	1.9
<i>Cercospora capsici</i>	24.8	0	0	0	0	0	0	2.1	1.7	0.5	0.5	0	0	0	0	0	0	0	0	1.5	0.3	0	0	0	0
<i>Colletotrichum capsici</i>	63.5	0	0	0	0	0	0	1.9	0.5	0	0.9	0.2	0	0	0	0	0	0	0	0.9	0	0	0.3	0	0
<i>Drechslera hawaiiensis</i>	27.4	0	0	0	1	0	0	0.6	0	0	1	0.2	0	0	0	0	0	0	0	0	0	0	0.3	0	0
<i>Fusarium oxysporum</i>	48	0	0	0	0	0	0	4.2	2.5	1.1	2.1	1.2	0.5	0	0	0	0	0	0	3.1	1.7	0.2	1.7	0.9	0.1
<i>F. solani</i>	51.6	0.7	0	0	4.3	1.1	0	2.2	1.5	0.6	2.3	1.6	0.2	0	0	0	2.1	0.2	0	1.7	0.9	0.1	1.9	1.1	0.4
<i>Macrophomina phaseolina</i>	37.5	1.1	0.1	0	0	0	0	3.1	2.5	1.2	1	0.2	0	0.4	0	0	0	0	0	2.1	1.7	0.2	0.5	0	0
<i>Phytophthora capsici</i>	33.2	3.2	1.1	0.5	0	0	0	1	0.2	0	1.6	1.1	0.3	1.9	0.2	0	0	0	0	0.6	0	0	1.1	0.2	0
<i>Pythium sp.</i>	22	0	0	0	0	0	0	1.1	0.4	0	1.9	0.7	0.1	0	0	0	0	0	0	0.7	0	0	1.3	0.2	0
<i>Rhizoctonia solani</i>	35	1.3	0	0	0	0	0	1.2	0.5	0.1	1.2	0.3	0	0.7	0	0	0	0	0	0.5	0	0	0.5	0	0
Mean	41.84	1.28	0.60	0.30	2.76	1.53	0.49	2.87	1.72	0.72	3.72	1.77	0.54	0.68	0.22	0.08	1.41	0.62	0.06	1.96	0.81	0.26	2.92	1.22	0.40

Table 3: Percentage occurrence of fungi after treatment with different fungicides alone and together with chitin by Agar plate method

Name of Fungi	Control	Aliette 80% WP			Antracol 70%			Score 250 EC			Cabrio Top 60% WDG			Aliette 80% WP+Chitin			Antracol 70% +Chitin			Score 250 EC+Chitin			Cabrio Top 60% WDG+Chitin		
		0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
<i>Alternaria alternata</i>	41	0.7	0.1	0	4.7	1.9	0.2	2.7	1.5	1.1	6.1	2.7	1.3	0	0	0	0.6	0	0	0.9	0.2	0	2.5	1.7	0.3
<i>A. solani</i>	37.1	0.5	0	0	1.7	0.5	0	2.3	1.9	0.5	1.9	0.7	0	0	0	0	0	0	0	0.7	0	0	0.9	0.1	0
<i>Aspergillus flavus</i>	70.2	3.7	1.7	0.1	8.1	6.2	4.3	8.3	5.2	2.9	9.9	4.9	0.2	0.2	0	0	1.9	0.3	0	4.2	1.6	0.3	6.5	2.2	1.1
<i>A. niger</i>	67.7	7.3	6.4	3.5	19.5	13	4.5	9.1	6.2	2.9	17.5	9.6	2.5	2.1	1.3	0.1	7.6	3.1	2.9	5.1	1.4	0.2	13.2	6.5	1.5
<i>Cercospora capsici</i>	28.3	0.4	0	0	1.7	0.2	0	2.7	2.1	1.6	0.5	0.7	0	0	0	0	0	0	0	0.5	0	0	0.3	0	0
<i>Colletotrichum capsici</i>	64.2	0.3	0	0	0.3	0	0	2.5	1.9	0.7	2.3	1.1	0.2	0.5	0	0	1.5	0.1	0	0.1	0	0	0.6	0	0
<i>Drechslera hawaiiensis</i>	31.3	0	0	0	1	0.2	0	1.3	0.1	0	1.5	0.1	0	0	0	0	1.3	0.2	0	0.3	0	0	0.9	0	0
<i>Fusarium oxysporum</i>	42	0.2	0	0	0.3	0	0	5.6	2.1	1.3	3.1	2.2	1.5	0.2	0	0	0.4	0	0	2.1	0.9	0	1.2	0.4	0
<i>F. solani</i>	55.2	0.7	0	0	2.3	0.9	0.2	2.7	1.6	0.5	2.7	1.9	0.5	0	0	0	1.2	0.3	0	0.8	0.1	0	1.3	0.5	0
<i>Macrophomina phaseolina</i>	39	1.5	0	0	0	0	0	4.1	2.7	1.5	1.5	0.5	0	0.1	0	0	0	0	0	1.5	0.3	0	0.1	0	0
<i>Phytophthora capsici</i>	35.4	3.3	1.2	0.7	0.5	0	0	1.5	0.4	0	2.1	1.9	0.3	0.5	0	0	0	0	0	0	0	0	0.5	0	0
<i>Pythium sp.</i>	27.1	0	0	0	0.7	0	0	1.9	0.6	0.1	2.3	1.2	0.3	0.3	0	0	0.2	0	0	0.4	0	0	0.7	0.2	0
<i>Rhizoctonia solani</i>	37.2	1.5	0.3	0	0.1	0	0	1.7	0.9	0.2	1.7	0.6	0	0	0	0	0	0	0	0.2	0	0	0	0	0
Mean	44.28	1.55	0.75	0.33	3.15	1.78	0.71	3.57	2.09	1.02	4.08	2.16	0.52	0.30	0.10	0.01	1.13	0.31	0.22	1.29	0.35	0.04	2.21	0.89	0.22

Table 4: Mean and Standard error of occurrence of fungi in fungicides together with chitin in Standard Blotter paper method

Treatments	0%	0.10%	0.20%	0.30%
Aliette 80% WP + Chitin	41.84±4.39	0.68±0.34	0.22±0.16	0.08±0.08
Antracol 70% + Chitin	41.84±4.39	1.41±0.89	0.62±0.49	0.06±0.05
Score 250 EC + Chitin	41.84±4.39	1.96±0.55	0.81±0.27	0.26±0.15
Cabrio Top 60% WDG + Chitin	41.84±4.39	2.92±1.36	1.22±0.64	0.4±0.21

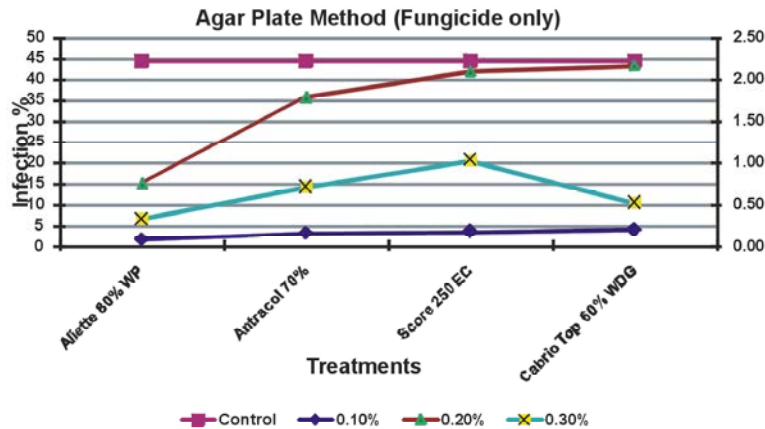


Fig. 1: Effect of different fungicides on the growth of fungi in Agar plate method

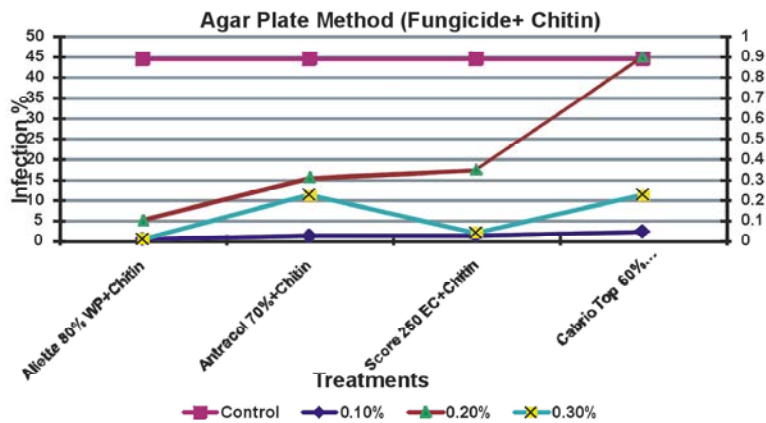


Fig. 2: Effect of different fungicides with chitin on the growth of fungi in Agar plate method

Table 5: F- ratios derived from ANOVA in Standard Blotter paper method for infection % of fungicides alone and with chitin on different concentrations against pathogenic fungi.

Source	F-ratio	P-value	LSD _{0.05}
Fungicides alone and with chitin	10.11	.0000***	0.53
Concentrations	65.06	.0000***	0.32
Fungicides with chitin × Concentrations	1.76	.0727ns	

F= F-ratio was obtained from ANOVA tables, LSD=Least significant difference at P=0.05

Table 5 shows the results of ANOVA using Blotter paper method for infection percentages in fungicide treatments alone and together with chitin in 0.10%, 0.20% and 0.30% concentrations when used for treatment against pathogenic fungi. Fungicide treatments showed significant differences. Likewise all concentrations also showed significant variation and inhibited the growth of all pathogenic fungi to varied extent. However, the interaction of fungicides and the concentrations was found significant.

Results of all fungicides alone in agar plate method showed the suppression of growth of all tested fungi, in which out of four fungicides tested Alliette (80% WP) at

0.30% concentration controlled the fungi. Whereas, 0.20% dose also reduced the growth of all fungi. Whenever Cabrio Top (60% WDG) at 0.30% also controlled all isolated fungi as compare same doses of Antracol (70%) and Score (250 EC) (Table 6). The infection % and treatment of fungicides only on the growth of isolated fungi by Agar plate method are shown in Figure 3.

The results of test fungicides with chitin in agar plate method showed the suppression of fungal growth compared to that when fungicides were used alone, in which out of all fungicides with chitin Alliette (80% WP) at 0.30% concentration controlled the fungi most.

Table 6: Mean and Standard error of the occurrence of fungi in fungicide treatments alone by Agar plate method

Treatments	0%	0.10%	0.20%	0.30%
Aliette 80% WP	44.28±4.14	1.55±0.58	0.75±0.49	0.33±0.27
Antracol 70%	44.28±4.14	3.15±1.50	1.78±1.06	0.71±0.46
Score 250 EC	44.28±4.14	3.57±0.71	2.09±0.49	1.02±0.28
Cabrio Top 60% WDG	44.28±4.14	4.08±1.31	2.16±0.71	0.52±0.21

Table 7: Mean and Standard error of occurrence of fungi in fungicides together with chitin in Agar plate method

Treatments	0%	0.10%	0.20%	0.30%
Aliette 80% WP + Chitin	44.28±4.14	0.3±0.16	0.1±0.1	0.01±0.01
Antracol 70% + Chitin	44.28±4.14	1.13±0.57	0.31±0.23	0.22±0.22
Score 250 EC + Chitin	44.28±4.14	1.29±0.45	0.35±0.16	0.04±0.03
Cabrio Top 60% WDG + Chitin	44.28±4.14	2.21±1.03	0.89±0.51	0.22±0.14

Table 8: F- ratios derived from ANOVA in Agar plate method for infection % of fungicides alone and with chitin on different concentrations against pathogenic fungi..

Source	F-ratio	P-value	LSD _{0.05}
Fungicides alone and with chitin	17.45	0.001***	0.55
Concentrations	56.48	0.001***	0.33
Fungicides with chitin × Concentrations	2.69	0.01**	

F= F-ratio was obtained from ANOVA tables, LSD=Least significant difference at P=0.05

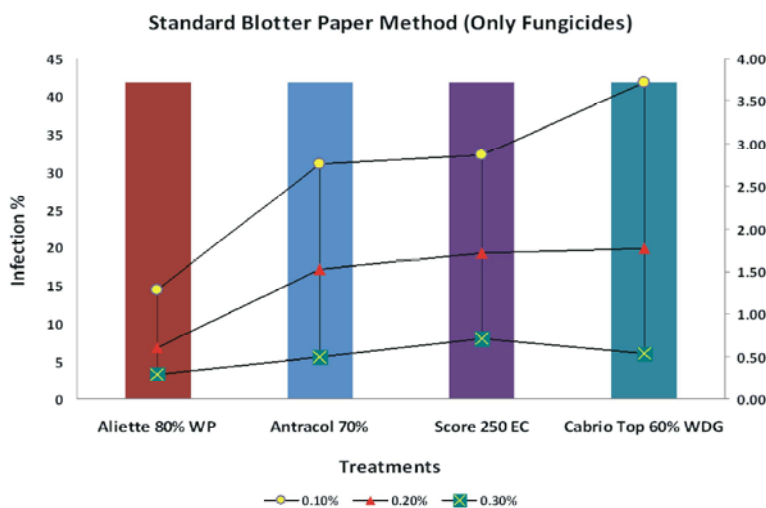


Fig. 3: Effect of different fungicides on the growth of fungi in Standard blotter paper method

Whereas, 0.20% dose also reduced the growth of all fungi significantly (P at the most 0.01). Whenever, Score (250 EC) at 0.30% also controlled all isolated fungi as compare to same doses of Cabrio Top (60% WDG) and Antracol (70%) (Table 7). The infection % and treatment of fungicides with chitin on the growth of isolated fungi by Agar plate method are shown in Figure 4.

Table 8 shows the results of ANOVA in Agar plate method for infection % of fungicides alone and with chitin in 0.10%, 0.20% and 0.30% concentrations were treated against pathogenic fungi. All concentrations showed

significant differences and inhibited the growth of all pathogenic fungi. Statistical analysis of fungicides alone and with chitin showed that Alliete (80% WP) at 0.30% concentrations showed significant inhibition of the growth followed by Antracol (70%), Cabrio Top (60% WDG) and Score (250 EC) that suppressed the mycelial growth of pathogenic fungi as compared to other treatments in Agar plate method. The result indicated that out of these fungicides; Alliete (80% WP) alone and with chitin at 0.20 and 0.30% were observed to be more effective and are recommended for strong fungicidal activity towards isolated pathogenic fungi.

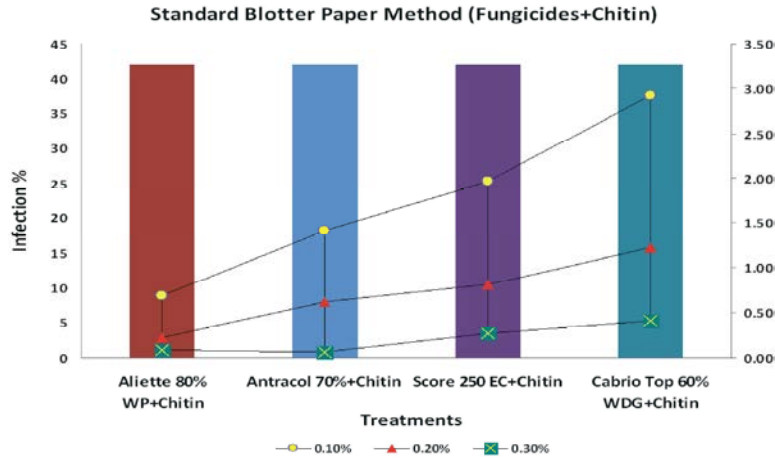


Fig. 4: Effect of different fungicides with chitin on the growth of fungi in Standard blotter paper method

Fungicides are routinely or frequently applied to control fungal pathogens (*Colletotrichum*, *Botrytis cinerea*, *Rhizoctonia solani*, *Phytophthora*, *Aletrnaira*, *Fusarium*, *Verticillium* and some other species) of chilli and tomato plants [53, 54]. Kuchitsu *et al.* [34], Bell *et al.* [35], Sneh and Henis [36] and Abd-El-Kareem [42] reported that chitin and chitosan are safe material to induce resistance against soil-borne and seed borne fungi and showed inhibitory effects against fungal pathogens. On the other hand, inhibitory effect of chitosan, a closely similar compound, against pathogenic fungi has been reported by Hirano *et al.* [37] and Abd-El-Kareem [42]. During the rainfall in summer season, chilli plants become infected by root, stem, leave and fruit are most frequently attacked by fungal pathogens. According to Matheron and Call [55], some treatments of fungicide Aliette and Fluazinam as well as Ridomil tended to reduce the incidence of disease in this trial. However, the high variability in disease incidence among the replicates of each treatment prevented the demonstration of statistically significant difference. Matheron and Porchas [56] investigated that the comparative efficacy of azoxystrobin, dimethomorph, Aliette and fluazinam as potential chemical management tools for root rot and stem rot of chilli plants has been demonstrated. Ghaffar [57] utilized various chemicals including Agrosan, Furadan, Benomyl and Captan to control the root rot fungi with different concentrations. These results to some extent supported by those obtained by Tripathi *et al.* [58] and Sitara and Hassan [59] on chilli seeds by Standard Blotter paper method and Deep freezing method. Sitara and Akhter [60] reported that fungicide Aliette showed better results as compare to Mancozeb and Thiophonate methyl at same doses. Sitara and Hassan

[59] also analyzed that Metalexyl + Mancozeb, Derosol, Copper Oxychlorite and Antracol were not effective @ 0.5% concentration whereas Mancozeb, Aliette and Ridomyl Gold reduced infection percent at same dose level.

It could be concluded from this research that fungicide Alliete (80% WP) alone or with chitin was found effective and showed significant inhibition of the growth against pathogenic fungi as compared to other fungicides. There results confirm those reported by Hirano *et al.* [37, Abd-El-Kareem [42], Tripathi *et al.* [58], Sitara and Akhter [60] and Sitara and Hassan [59].

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