



Effect of Bion, Amistar and Vitavax on anthracnose of chilli

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Received 11 January 2004, accepted 28 April 2004.

Abstract

Seeds of Mymensingh local and Pabna local varieties were used for seed treatment with Bion (as inducer of resistance) @ 0.005, Amistar (as fungicide) @ 0.05% and 0.1% and Vitavax-200 (as fungicide) @ 0.3% of seed weight. The percent germination was highest in Amistar and Vitavax-200-treated seeds and these chemicals most effectively controlled seed borne fungi of chilli. All the tested chemicals reduced the seedling mortality over control. The results revealed that Bion reduced the seedling mortality of chilli variety Mymensingh local and Pabna local by 87.3% and 100% respectively over control. The results showed that plants raised from Bion-treated seeds did not show die-back symptom but this symptom was common in plants raised from Amistar and Vitavax-200-treated seeds. Lesion size, percent leaf infection and percent leaf area damaged were also observed less in plants raised from Bion and Amistar-treated seeds as compared to the other treatments. Fruits of the plants raised from Bion-treated seeds showed moderately resistance reaction against anthracnose whereas fruits of the plants raised from Amistar and Vitavax-treated seeds showed susceptible to highly susceptible reaction against anthracnose of chilli.

Key words: Chilli, seed treatment, anthracnose, Bion, Amistar, Vitavax-200.

Introduction

Chilli (*Capsicum annuum* L.) is one of the most important spice crops in the world and grown in all seasons and areas of Bangladesh. The average yield of chilli is 0.042 t ha⁻¹ which is very low as compared to the yield of other chilli growing countries of the world¹. There are many factors responsible for the low yield of the crop. Among the various factors diseases are predominant. Fungal diseases play a vital role in reducing the yield of the crop. Out of the fungal diseases, anthracnose incited by *Colletotrichum capsici* is very important, because it inflicts a considerable quantitative and qualitative losses of the crop in the field as well as in storage. Management strategies reflect use of presumed disease free seeds and seedlings, resistant cultivars and fungicidal sprays. All approaches are not fully successful but have their disadvantages, such as the brief commercial life of resistant cultivars or occurrence of fungicide resistance². Therefore, there is a constant requirement for new genetic resistance and for new class of chemicals to continue the fight against diseases. Plant activators may induce systemic resistance in plants. Induced systemic resistance (ISR) may be defined as upon irritation of a plant tissue by microorganisms or chemicals, a signal of yet unknown nature is released. This and/or a second signal are then translocated to untreated tissues or plant parts. There it conditions and intensifies a resistance response, when these parts are challenged by inoculation with plant pathogens²⁶. It was demonstrated that various chemical agents such as salicylic acid²⁹, 2,6-di-chloro-isonicotinic acid²⁰ and benzothiadiazole² can induce systemic resistance. Among the various chemicals only few have reached commercialization. Bion has been reported to induce resistance in wheat and rice against fungal pathogens^{2, 8, 21}, in bean and cucumber against bacterial and fungal infections^{23, 27} and in tobacco and *Arabidopsis* spp. against fungal, bacterial and viral infections^{6, 9, 17}. Bion compiles with the definition of SAR (systemic activated resistance) inducer; it gives protection to some spectrum of pathogens, causes the expression of the same

molecular and biochemical markers (e.g. pathogenesis related proteins) as biological inducers and does not have direct antimicrobial activity¹⁵. Resistance inducers used in conjunction with or alternated with traditional fungicides and bactericides may lead to a reduction of the number of applications and perhaps dose rate not as necessarily replacement^{19, 24}. Therefore, the risk of development of SAR-insensitive strains seems very low because several mechanisms appear to be activated simultaneously against the pathogen attack during SAR expression. In light of the above facts, the present research work has been undertaken to determine the comparative efficacy of Bion (0.005%) as inducer with fungicides Amistar (0.05 and 0.1%) and Vitavax-200 (0.3%) against anthracnose (*Collectotichum capsici*) of chilli.

Materials and Methods

Collection and storing of seeds: The experiments were conducted both in the laboratory and greenhouse of Seed Pathology Centre, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. Healthy and diseased ripen chilli fruits of 2 local varieties viz. Pabna local and Mymensingh local were randomly collected from farmers field located in 5 different Upazillas of two districts viz. Pabna and Mymensingh during the period of February to March 2001. The collected fruits were sun-dried, crushed and the seeds were collected. The seeds were then kept in brown paper bags with proper label and stored in the refrigerator at 5°C for subsequent studies.

Determination of efficacy of Bion, Amistar and Vitavax-200 on germination and seed-borne fungi

Blotter method of seed health test: A composite seed sample of each variety was assayed for the presence of seed borne fungi by standard blotter method¹³. Three pieces of 9 cm filter paper (Whatman No.1) were soaked in distilled water and placed at the

bottom of a plastic Petri dish. Two hundred seeds from each of the samples were taken randomly and then placed on the moist filter paper in 8 Petri dishes (25 seeds per plate). The Petri dishes were then incubated at 20°C, alternating cycles of near ultraviolet light and darkness (12 h/12 h) in the incubation room for 14 days. After incubation, the seeds were examined for the presence of seed-borne fungi and identified by observing their growth characters on the incubated seed on blotter under stereomicroscope at 25x magnification^{16,25}.

Chemicals used and their doses: The chemicals namely Bion (benzothiadiazole) 0.005%, Amistar (azoxystrobin) 0.05% and 0.1% and Vitavax-200 (carboxin) 0.3% were used as treatments including a control (only water).

Efficacy of Bion, Amistar and Vitavax-200 on seed-borne fungi
Seed treatment followed by blotter method: The chilli seeds of Mymensingh local and Pabna local varieties were used for the seed treatment based on the prevalence of seed-borne fungi of collected varieties. Two hundred seeds of each selected samples were soaked in solutions of Bion 0.005% and Amistar (0.05% and 0.1%) separately and in water for the control treatment for 12 hours. Then the treated seeds were plated on moistened filter paper at the rate of 25 seeds per plate in 8 replications. The test was carried out following the method of international rules for seed health testing¹³. After incubation, the germinated seeds were examined under stereomicroscope for detecting the fungal growth over the seeds on blotter under different treatments.

Tray method of determining efficacy of Bion, Amistar and Vitavax-200: 200 seeds of each selected samples were treated separately with Bion, Amistar and Vitavax-200 following the same method as of determination of efficacy in controlling seed borne fungi and were sown in plastic trays containing sterilized soil at the rate of 100 seeds per tray in nethouse. One control treatment of each sample was also maintained. Data were recorded on seed germination and seedlings mortality. The results were expressed in percentage.

Pot experiment

Preparation of soil: Soil and well-decomposed cowdung were collected and mixed (4:1) very finely. The mixed soil was sterilized with formalin (37%) at the rate of 24.35 ml dissolved in water, filled to the volume 300 ml and added to soil (1 cft). The treated soil was covered by polythene sheet for 72 hours and after that the polythene sheet was removed and the soil was exposed for 48 hours in order to remove excess vapour of formalin. Plastic trays and earthen pots were then filled with sterilized soil.

Raising of seedlings: Seeds were treated as per treatment and sown in plastic trays (32 cm × 22 cm). 200 seeds per tray were sown and proper cares were maintained. Seedlings of the trays obtained from healthy seeds of both varieties of each treatment were used for transplanting in pots.

Transplantation and care of seedlings: Healthy and uniform-sized seedlings (40 days old) obtained from healthy seeds of both varieties of each treatment were uprooted carefully from the plastic tray and transplanted two seedlings in each pots (25 cm diameter)⁵. Watering of the seedlings was done in every morning for the first seven days after transplantation for the

establishment of the seedlings. Malathion 57EC was sprayed @ 0.2% to control chilli aphid at flowering stage (80 days old plants).

Isolation and culture of *Colletotrichum capsici*: The organism *Colletotrichum capsici* isolated from the seed by observing fruiting structure (Acervuli) was cultured in PDA. For obtaining pure culture, conidia of *Colletotrichum capsici* were transferred from germinated seed surface to PDA plate with the help of a fine-pointed sterile needle. After growth of the fungal colony, the hyphal tip was transferred to another PDA plate and incubated at 28°C for luxuriant growth. Mycelial blocks (8 mm diameter) were taken by using block cutter. Fourteen days old cultures of the pathogen grown on PDA medium were used for preparing conidial suspension. The conidia were taken off from the surface of PDA with the help of a sterile camel hair brush and transferred to a beaker. The resultant conidial suspension was filtered through a double layered cheese cloth and then one drop of Tween-20 was added. The conidial suspension (1×10^4 conidia ml⁻¹) was prepared.

Inoculation test: Stem and shoots of 120 days old plants were inoculated with mycelial block by pinpricked and unpricked method. The injury on stem and shoots was made with the help of sterile needle. Inoculum was put on the point of inoculation and moistened small pad of absorbent sterile cotton was kept on it. Cotton was removed after 24 hours of inoculation. Another set of inoculation was done by unpricked method. Data were collected on lesion length and symptomatic condition of the shoot.

Leaves of 120 days old plants were inoculated by pinpricked and unpricked method with conidial suspension (1×10^4 conidia ml⁻¹) of *Colletotrichum capsici* by spraying with self-compressed hand sprayer. Another set of study of inoculation by unpricked method was done. Both pinpricked and unpricked inoculated plants were incubated under humid conditions for 24 hours by keeping them covered with polythene sheets at 28°C. Data were recorded on per cent leaf infection and percent leaf area damaged.

Mature green fruits from each treatment of both the varieties were taken, surface sterilized with 0.1% mercuric chloride, washed with sterilized water (three times) and pricked lightly with sterilized needle and with conidial suspension (1×10^4 conidia ml⁻¹) of *Colletotrichum capsici*. The inoculated fruits were placed in moist chambers at 28±2°C. For each study control (pricked fruits without inoculation) was maintained. The extent of development of lesions on the fruits were measured. Ripe fruits from all the treatments of both the varieties were inoculated following the same method as of green fruit inoculation. Observations were taken on degree of fruit rot and the extent of development of lesions on the fruit was measured.

Scoring of disease severity: The disease reaction was scored on the basis of 0-4 scale²⁸ where tolerant (I)=0% infection (area), resistant (R)=1-5% infection, moderately resistant (MR)=5.1-25% infection and, susceptible (S)=25.1-50% infection. Moreover, percent diseased fruits was also graded following the grade as used by Basak² where tolerant (I) = no disease, resistant (R) = 1-5% diseased fruits, moderately resistant (MR)= 6-25% diseased fruits, susceptible (S)= 26-50% diseased fruits and highly susceptible (HS)=51% and above diseased fruits. The data on different parameters were subjected to analyses and treatment means were compared by least significance difference (LSD) test.

Results

Effect of Bion, Amistar and Vitavax-200 on germination and seed-borne fungi

Seeds obtained from healthy fruits: Highest percent (96%) germination of Mymensingh local variety was recorded both in T₃ (Amistar, 0.05%) and T₄ (Amistar, 0.1%) followed by T₅ (Vitavax-200, 0.3%), while the lowest percent germination (52%) was recorded in T₂ (Bion, 0.05%) which was significantly different from all other treatments (Table 1). Maximum percent germination (77%) of Pabna local variety was obtained both in T₃ (Amistar, 0.05%) and T₄ (Amistar, 0.1%) and minimum percent germination (29%) was recorded in T₂ (Bion, 0.005%). Seed-borne infection of Mymensingh local variety due to *Colletotrichum capsici*, *Curvularia lunata* and *Fusarium* spp. were 0.5%, 1.0% and 1.0% respectively in control treatments, while other treatments resulted the complete inhibition of these fungi. The seed-borne infections of Pabna local variety were 0.5, 0.5 and 2.0% by *Colletotrichum capsici*, *Curvularia lunata* and *Fusarium* spp., respectively in control treatment, but all other treatments completely inhibited the growth of these fungi except T₂ (Bion, 0.005%) that yielded 1.0% *Fusarium* spp.

Seeds obtained from diseased fruits: The germination of seeds of Mymensingh local variety ranged from 13-40%. The highest percent germination (40%) of seeds was recorded when seeds were treated with Vitavax (T₅) followed by T₃ (Amistar, 0.1%) while T₂ (Bion) resulted the lowest percent germination (13%) (Table 2). The control treatment resulted maximum seed-borne infection (12%) by *Colletotrichum capsici* and *Fusarium* spp., while minimum infection (2.5%) was observed in the case of *Cercospora capsici*. The results revealed that all chemicals appeared to be effective in inhibiting the growth of seed-borne fungi. The treatment T₂ (Bion, 0.005%) significantly reduced the growth of all the fungi compared to the untreated control. T₄ (Amistar, 0.1%) and T₅ (Vitavax-200, 0.3%) completely controlled seed borne fungi but T₃ (Amistar, 0.05%) inhibited the growth of all the fungi completely except *Colletotrichum capsici* and *Fusarium* spp.

The germination of seeds of Pabna local variety ranged from 25-46%. The highest percent germination of seeds (46%) was recorded in T₃ (Amistar, 0.05%) followed by T₄ (Amistar, 0.1%) and T₅ (Vitavax-200, 0.3%). In controlling the growth of seed-borne fungi all chemicals appeared to be effective in comparison with the untreated seeds. No seed-borne infection was found in T₂ (Bion, 0.005%) in case of fungi *Curvularia lunata*, while maximum (4%) infection was found by *Colletotrichum capsici* followed by *Fusarium* spp. The control treatment showed maximum (16%) infection by *Fusarium* spp. and minimum infection by *Curvularia lunata*. T₃ (Amistar, 0.05%) completely inhibited seed infection by the fungi *Alternaria tenuis*, *Cercospora capsici* and *Curvularia lunata*. On the contrary, T₄ (Amistar, 0.1%) and T₅ (Vitavax-200, 0.3%) completely retarded the growth of all the genera studied. T₂ (Bion, 0.05%) and T₃ (Amistar, 0.05%) did not inhibit completely the growth of *Colletotrichum capsici* and *Fusarium* spp.

Effect of Bion, Amistar and Vitavax-200 on germination and seedling mortality in tray experiment

Seeds obtained from healthy fruits: Germination of seeds of Mymensingh local variety under different treatment did not show any significant variation though it ranged from 89.5-94%

(Table 3). T₃ (Amistar, 0.05%), T₄ (Amistar, 0.1%) and T₅ (Vitavax-200, 0.3%) showed 94% germination which resulted 1.07% increase in germination over control, whereas T₂ (Bion, 0.005%) showed 89.5% germination that resulted 3.76% reduction in germination over control. The germination of seeds of Pabna local variety did not vary from one treatment to another though the germination ranged from 63-75% (Table 3). The highest percent of seed germination recorded both in T₃ (Amistar, 0.005%) and T₅ (Vitavax-200, 0.3%) that indicated 11.94% increase of seed germination over control. The lowest percent of germination (63%) was recorded in T₂ (Bion, 0.005%) which resulted 5.97% decrease in germination over control. Seedling mortality of both varieties was zero when seeds collected from the healthy fruits.

Seeds obtained from diseased fruits: The highest percent of seed germination of Mymensingh local variety (47%) was recorded in T₃ (Amistar, 0.05%) which resulted 30.55% increase over control, while the lowest percent germination (33%) was obtained in seeds treated with T₂ (Bion, 0.005%) that resulted reduction (8.33%) in germination over control. The other treatments showed the remarkable increase of germination over control treatment as well as T₂ (Bion, 0.005%). The germination of seeds of Pabna local variety ranged from 33.5-49.0%, while T₃ (Amistar, 0.05%) and T₅ (Vitavax-200, 0.3%) showed the maximum percentage of germination resulting 22.5% increase over control. The minimum percentage of germination (33.5%) was recorded in T₂ (Bion, 0.005%) that indicated 16.25% reduction in germination over control.

All the treatments employed in the study showed significant effect in respect of seedling mortality when seeds of diseased fruits for both varieties were used (Table 3). In the case of Mymensingh local variety, the highest percent of seedling mortality (12.44%) was recorded in T₁ (control) while the lowest percent (1.58%) was found in T₂ (Bion, 0.005%) and the other treatments showed significant decrease of seedling mortality over control. In Pabna local variety the maximum (10.0%) mortality of seedlings was observed in control treatment while mortality was zero in T₂ (Bion, 0.005%) that resulted 100% reduction in germination over control. The mortality of seedlings was also lower in other treatments as compared to control.

Effect of Bion, Amistar and Vitavax-200 on anthracnose of inoculated fruits

Mature green fruits: The visible symptoms of anthracnose developed only after 3 days of inoculation in T₁ (control) and T₅ (Vitavax-200, 0.3%), while in T₂ (Bion, 0.045%) visible symptoms appeared after 6 days of inoculation in both Mymensingh and Pabna local varieties (Table 4). The other two treatments resulted the symptoms after 4 days of inoculation in both Mymensingh and Pabna local variety. The highest lesion size viz. 35.5 mm and 29.1 mm, respectively were recorded in Mymensingh local variety and Pabna local variety under control treatment while the lowest lesion size viz. 10.2 mm and 8.5 mm respectively were recorded in T₂ (Bion, 0.005%) both in Mymensingh and Pabna local variety.

Ripe fruits: The visible symptoms of anthracnose in Mymensingh local variety was appeared only after 2 days of inoculation in case of T₁ (control) and T₅ (Vitavax-200, 0.3%), while after 5 days of inoculation in T₂ (Bion, 0.005%) as shown in (Table 5). After 10 days of inoculation lesion length was highest (38.56 mm) in T₁

Table 1. Effect of seed treatment with Bion, Amistar and Vitavax-200 on germination and seed-borne fungi of chilli (seeds obtained from healthy fruits)

Treatment	% Seed-borne fungi		
	<i>Colletotrichum capsici</i>	<i>Curvularia lunata</i>	<i>Fusarium</i> spp.
Mymensingh local variety			
T ₁ =Control (untreated)	91.0	1.0	1.0
T ₂ =Bion (0.005%)	52.0	0.0	0.0
T ₃ =Amistar (0.05%)	96.0	0.0	0.0
T ₄ =Amistar (0.1%)	96.0	0.0	0.0
T ₅ =Vitavax-200 (0.3%)	94.0	0.0	0.0
Pabna local variety			
T ₁ =Control (untreated)	59.0	0.5	2.0
T ₂ =Bion (0.005%)	29.0	0.0	1.0
T ₃ =Amistar (0.05%)	77.0	0.0	0.0
T ₄ =Amistar (0.1%)	77.0	0.0	0.0
T ₅ =Vitavax-200 (0.3%)	75.0	0.0	0.0

200 × 3 seeds were examined for each treatment, NS= Not significant

Table 2. Effect of seed treatment with Bion, Amistar and Vitavax-200 on germination and seed-borne fungi of two varieties of chilli seeds obtained from diseased fruits.

Treatment	% Germination	% Seed-borne fungi			
		<i>Alternaria tenuis</i>	<i>Cercospora capsici</i>	<i>Colletotrichum capsici</i>	<i>Curvularia lunata</i>
Mymensingh local variety					
T ₁ =Control (untreated)	26.00	5.00	2.50	12.00	3.50
T ₂ =Bion (0.005%)	13.00	2.50	1.00	5.00	1.00
T ₃ =Amistar (0.05%)	31.00	0.00	0.00	0.50	0.00
T ₄ =Amistar (0.1%)	38.00	0.00	0.00	0.00	0.00
T ₅ =Vitavax-200 (0.3%)	40.00	0.00	0.00	0.00	0.00
Pabna local variety					
T ₁ =Control (untreated)	35.00	4.00	3.00	12.00	2.00
T ₂ =Bion (0.005%)	25.00	1.00	0.50	4.00	0.00
T ₃ =Amistar (0.05%)	46.00	0.00	0.00	1.00	0.00
T ₄ =Amistar (0.1%)	45.00	0.00	0.00	0.00	0.00
T ₅ =Vitavax-200 (0.3%)	45.00	0.00	0.00	0.00	0.00

Data based on 200 × 3 seeds for each treatment

Table 3. Effect of seed treatment with Bion, Amistar and Vitavax-200 on germination and seedling mortality of chilli (tray experiment).

Treatment	Seeds from healthy fruits		Seeds from diseased fruits	
	% Seed germination	% seedling mortality	% Seed germination	% seedling mortality
Mymensingh local variety				
T ₁ =Control (untreated)	93.0	0.00	36.0cd	12.44 a
T ₂ =Bion (0.005%)	89.5 (-3.76)	0.00	33.0d (-8.33)	1.58 b (-87.30)
T ₃ =Amistar (0.05%)	94.0 (1.07)	0.00	47.0 a (30.55)	2.12 b (-82.93)
T ₄ =Amistar (0.1%)	94.0 (1.07)	0.00	40.0 bc (11.11)	2.32 b (-81.35)
T ₅ =Vitavax-200 (0.3%)	94.0 (1.07)	0.00	41.0 b (13.88)	2.44 b (-80.38)
LSD (P=0.05)	NS		4.234	2.617
Pabna local variety				
T ₁ =Control (untreated)	67.0	0.00	40.0 ab	10.00 a
T ₂ =Bion (0.005%)	63.0 (-5.97)	0.00	33.5 b (-16.25)	0.00 c (-100.0)
T ₃ =Amistar (0.05%)	75.0 (11.94)	0.00	49.0 a (22.50)	1.01 bc (-89.9)
T ₄ =Amistar (0.1%)	70.0 (4.48)	0.00	43.0 ab (7.50)	1.04 bc (-89.60)
T ₅ =Vitavax-200 (0.3%)	75.0 (11.94)	0.00	49.0 a (22.50)	2.11 b (-78.90)
LSD (P=0.05)	NS		9.989	1.558

200 × 3 seeds were examined for each treatment
Data in parentheses indicate percent increase or decrease over control.
ML= Mymensingh local, PL= Pabna local, NS= Not significant

Table 4. Effect of seed treatment with Bion, Amistar and Vitavax-200 on anthracnose of inoculated mature green fruits of Mymensingh local and Pabna local variety.

Treatment	Number of days until appearance of visible symptoms	Lesion size (mm) after									
		3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days		
Mymensingh local variety											
T ₁ =Control (untreated)	3	4.20	6.90	7.20	9.10	13.20	19.20	27.20	35.50		
T ₂ =Bion (0.005%)	6	-	-	-	2.80	3.90	5.20	7.90	10.20		
T ₃ =Amistar (0.05%)	4	-	3.20	3.90	5.50	7.20	10.50	18.50	24.60		
T ₄ =Amistar (0.1%)	4	-	3.30	4.90	6.90	9.20	13.90	19.10	26.50		
T ₅ =Vitavax-200 (0.3%)	3	3.40	5.00	6.80	10.20	15.40	20.20	26.20	34.30		
Non-inoculated control (untreated)											
Pabna local variety											
T ₁ =Control (untreated)	3	3.80	4.20	5.00	6.70	10.20	18.50	23.20	29.10		
T ₂ =Bion (0.005%)	6	-	-	-	2.50	3.50	4.60	5.20	8.50		
T ₃ =Amistar (0.05%)	4	-	2.90	3.10	3.80	4.50	10.60	12.40	15.50		
T ₄ =Amistar (0.1%)	4	-	2.50	3.60	5.20	10.50	13.50	15.10	16.40		
T ₅ =Vitavax-200 (0.3%)	3	3.30	4.70	7.20	9.50	12.50	17.20	25.20	28.82		
Non-inoculated control (untreated)											
- = No symptom											

Table 5. Effect of seed treatment with Bion, Amistar and Vitavax-200 on anthracnose of inoculated ripe fruits of Mymensingh local and Pabna local variety.

Treatment	Number of days until appearance of visible symptoms	Lesion size (mm) after									
		2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	
Mymensingh local variety											
T ₁ = Control (untreated)	2	3.30	4.22	7.80	9.90	12.98	16.53	21.10	27.08	38.56	
T ₂ = Bion (0.005%)	5	-	-	-	2.80	3.60	3.80	5.80	8.30	11.80	
T ₃ = Amistar (0.05%)	3	-	3.20	4.00	5.20	7.45	9.15	12.50	17.00	25.65	
T ₄ = Amistar (0.1%)	4	-	-	2.63	7.63	10.76	17.03	20.33	25.66	27.76	
T ₅ = Vitavax-200 (0.3%)	2	3.33	3.43	4.13	7.40	11.37	15.40	20.50	26.30	35.40	
Non-inoculated control (untreated)											
Pabna local variety											
T ₁ = Control (untreated)	2	3.30	4.76	5.12	6.40	8.12	10.96	15.06	22.96	33.16	
T ₂ = Bion (0.005%)	5	-	-	-	3.00	3.50	4.80	5.30	7.90	10.20	
T ₃ = Amistar (0.05%)	4	-	-	2.25	3.50	6.35	8.90	12.85	16.50	19.12	
T ₄ = Amistar (0.1%)	4	-	-	2.75	2.85	5.10	7.85	10.80	14.80	18.80	
T ₅ = Vitavax-200 (0.3%)	2	3.10	3.15	5.00	6.10	6.90	9.25	13.75	20.90	31.35	
Non-inoculated control (untreated)											
- = No symptom											

Table 6. Effect of seed treatment with Bion, Amistar and Vitavax-200 on the development of die-back of chilli in Mymensingh local and Pabna local variety

Treatment	Stem inoculation by conidia of <i>Colletotrichum capsici</i> ($1 \Delta 10^4$)		Artificial inoculation of branch with mycelial block of <i>Colletotrichum capsici</i> (8mm diam.)	
	Lesion size (mm)		Lesion size (mm)	
	7DAI	14DAI	7DAI	14DAI
Mymensingh local variety T ₁ = Control (untreated)				
T ₂ = Bion (0.005%)	30.40 a	31.60	41.00	78.00
T ₃ = Amistar (0.05%)	25.50 b	28.76	28.20	29.50
T ₄ = Amistar (0.1%)	29.00 a	30.50	28.40	30.80
T ₅ = Vitavax-200 (0.3%)	29.30 a	30.33	30.00	32.00
LSD (P=0.05)	1.597	NS	32.00	34.00
Pabna local variety T ₁ = Control (untreated)				
T ₂ = Bion (0.005%)	28.62 a	34.00	39.50	60.00
T ₃ = Amistar (0.05%)	24.73 b	27.67	24.50	27.20
T ₄ = Amistar (0.1%)	27.83 a	33.20	33.50	34.50
T ₅ = Vitavax-200 (0.3%)	27.00 a	33.10	30.30	34.00
LSD (P=0.05)	28.10 b	33.83	34.00	37.60
	1.572	1.660	-	-

Data having same letter (s) are statistically identical
DAI= Days after inoculation, NS= Not significant.

Table 7. Effect of seed treatment with Bion, Amistar and Vitavax-200 on the incidence and reaction of leaf spot as well as anthracnose of chilli when artificially inoculated with conidial suspension of *Colletotrichum capsici* (pin-prick method).

Treatment	Leaf spot		Anthracnose on chilli fruit	
	% leaf infection ^x	% leaf area damaged ^y	% infection	% diseased fruits
Mymensingh local variety T ₁ =Control (untreated)	100.00	11.50	55.83	87.50
T ₂ = Bion (0.005%)	71.18	6.75	15.00	11.11
T ₃ = Amistar (0.05%)	78.00	8.30	33.30	40.00
T ₄ = Amistar (0.1%)	81.25	7.12	33.60	40.00
T ₅ = Vitavax-200 (0.3%)	100.00	11.50	53.66	71.42
Pabna local variety T ₁ =Control (untreated)	100.00	13.87	50.57	80.00
T ₂ = Bion (0.005%)	75.00	7.75	12.00	10.00
T ₃ = Amistar (0.05%)	81.25	11.12	29.33	33.39
T ₄ = Amistar (0.1%)	81.25	8.25	32.66	27.27
T ₅ = Vitavax-200 (0.3%)	96.87	11.25	52.75	77.27

X=32 leaves/treatments were inoculated by pin-pricked method spraying with conidial suspension

Y= Average damaged area (%) of 8 leaves.

MR= Moderately resistant, HS= Highly susceptible and S=susceptible

(untreated control) and lowest (11.8 mm) in T₂ (Bion, 0.005%). The first visible symptoms of anthracnose in Pabna local variety was recorded only after 2 days of inoculation in T₁ (control) and T₅ (Vitavax-200, 0.3%), while in T₂ (Bion, 0.005%) the first symptoms were found after 5 days of inoculation. Lesion length after 10 days of inoculation was maximum (33.16 mm) in T₁ (control) and minimum (10.2 mm) in T₂ (Bion, 0.005%).

Effect of Bion, Amistar and Vitavax-200 on development of die-back of chilli

In case of stem inoculation of Mymensingh local variety, the treatments used for the study showed significant difference in respect of lesion size 7 days after inoculation (DAI) but insignificant 14 days after inoculation (DAI) when stem inoculation was done (Table 6). At 7 DAI, significantly largest lesion (30.4 mm) was measured in T₁ (control) that was followed by T₃ (Amistar, 0.05%). Significantly smallest lesion (25.5 mm) was measured in T₂ (Bion, 0.005%). In case of 14 DAI, lesion size did not vary significantly among the treatments though it ranged from 28.76 mm to 31.60 mm.

In case of branch inoculation, the symptom of die-back appeared at 7 DAI on inoculated injured surfaces in all the treatments in Mymensingh local variety. The largest lesions viz. 41 and 78 mm, respectively were measured in control treatment (T₁) at 7 and 14 DAI, while the smallest lesions at 7 and 14 DAI were 28.2 and 29.5 mm respectively. Inoculated young shoots in all the treatments were withered and died except T₂ (Bion, 0.005%) and T₄ (Amistar, 0.1%) after inoculation at 7 DAI, but at 14 DAI shoots of the plants under all treatments died except in T₂ (Bion, 0.005%).

The largest size of lesions in Pabna local variety viz. 39.5 mm and 60.0 mm respectively were recorded in control treatment (T₁) at 7 DAI and 14 DAI, while the smallest lesions 24.5 mm and 27.2 mm, respectively were found in T₂ (Bion, 0.005%) both at 7 and 14 DAI. All the inoculated shoots in all the treatments were withered and died except T₂ (Bion, 0.005%) at 7 DAI.

Effect of Bion, Amistar and Vitavax-200 on the incidence and severity of leaf spot and anthracnose

The leaf spot symptom developed in inoculated injured leaves in both the varieties. The percent leaf infection under different treatments varied from 71.18 to 100% (Table 7).

The maximum leaf infection and leaf area diseased in Mymensingh local variety were recorded in T₁ (control) followed by T₅ (Vitavax-200, 0.3%), while minimum in T₂ (Bion, 0.005%). The highest percent infection (55.83%) and percent diseased fruits (87.5%) were recorded in T₁ (control) followed by T₅ (Vitavax-200, 0.3%), while the lowest counts were recorded in T₂ (Bion, 0.005%). The highly susceptible (HS) reaction was recorded in T₁ (control) and T₅ (Vitavax-200, 0.3%), whereas susceptible (S) reaction was found in T₃ and T₄, but moderately resistant (MR) reaction was found only under T₂ (Bion, 0.005%).

The highest percent of leaf infection and leaf area diseased in Pabna local variety were recorded in T₁ (control) while the lowest count was made in T₂ (Bion, 0.005%). The percent leaf infection and leaf area damaged were also lower in all other treatments compared to control treatment. The highest percent infection and percent diseased fruits were recorded in T₁ (control) followed by T₅ (Vitavax-200, 0.3%) while the lowest was recorded in T₂ (Bion, 0.005%). The rest of the treatments resulted comparatively lower percent of infection and percent diseased fruits. Regarding disease

reaction, it has been observed that the trend of disease reaction was similar as in Mymensingh local variety.

Discussion

In the present study eight different fungi viz. *Alternaria tenuis*, *Aspergillus* spp., *Cercospora capsici*, *Colletotrichum capsici*, *Curvularia lunata*, *Fusarium* spp., *Macrophomina phaseolina* and *Penicillium* spp. were found to be associated with the seeds obtained from diseased fruits of chilli. Five different seed-borne fungi such as *Aspergillus* spp., *Colletotrichum capsici*, *Curvularia lunata*, *Fusarium* spp. and *Penicillium* spp. were found to be associated with the healthy fruits of chilli. In Mymensingh and Pabna local varieties five different fungi viz. *Alternaria tenuis*, *Cercospora capsici*, *Colletotrichum capsici*, *Curvularia lunata* and *Fusarium* spp. were found to be associated with the diseased fruits, while three different fungi viz. *Colletotrichum capsici*, *Curvularia lunata* and *Fusarium* spp. were found in the healthy fruits. A considerable number of seed-borne fungal pathogens belonging to the genera *Alternaria*, *Aspergillus*, *Cercospora*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Macrophomina* and *Penicillium* have been detected in chilli seeds by many researchers^{2-4, 7, 11, 12}. The present findings clearly showed that *Alternaria tenuis*, *Aspergillus* spp., *Cercospora capsici*, *Colletotrichum capsici*, *Curvularia lunata* and *Fusarium* spp. associated with the tested seed samples significantly reduced germination. Similar results were also reported by earlier workers^{11, 14}. Percent germination in treated seeds was increased more than in untreated except in Bion-treated seeds as Bion is not a fungicide. It is an inducer of resistance that showed SAR in plants²⁶. Amistar (0.05% and 0.1%) and Vitavax-200 completely inhibited the growth of *Alternaria tenuis*, *Cercospora capsici* and *Curvularia lunata* in both chilli varieties when seeds were obtained from diseased fruits. Besides these, Amistar (0.1%) and Vitavax completely inhibited the growth of *Fusarium* spp. when seeds were obtained from healthy fruits. This result is in agreement with earlier results^{12, 22}. The results indicated that Bion is also effective in controlling the seed-borne fungi of chilli and stem infection. In our study Bion was more effective in reduction of seedling mortality and the incidence of leaf spot and die-back disease compared to control, Amistar (0.05 and 0.01%) and Vitavax-200. In Pabna local variety, no seedlings died and young shoots of the plants did not show die-back symptom when seeds were treated with Bion. In Mymensingh local variety, shoots of plants raised from seeds treated with Bion and Amistar (0.1%) did not show die-back though other treatments showed. Amistar and Vitavax-200 were also effective chemicals but their effect was not better than Bion in reducing seedling mortality and the incidence of leaf spot and die-back. The result proved that infection starts later on fruits in Bion treatment compared to the other treatments. Infection also starts later on green fruit than ripen fruits. The spread of lesion was slow in Bion treatment compared to other treatments, indicating the possibility of induction of resistance. The disease incidence was lower in Bion treatment compared to control and the other treatments. Fruits of plants raised from Bion-treated seeds showed moderate resistance reaction against anthracnose while fruits of the plants raised from control and Vitavax-200-treated seeds were highly susceptible. Fruits of the plants raised from Amistar-treated seeds showed susceptible reaction against anthracnose of chilli.

Conclusions

The results of the present study clearly demonstrated that plants raised from Bion-treated seeds did not show die-back symptom with less percent leaf area infection and percent area damaged compared to other chemicals used in the study. The findings of this investigation also revealed that fruits of the plants raised from Bion-treated seeds showed moderate resistance reaction against anthracnose whereas fruits of the plants raised from Amistar- and Vitavax-treated seeds showed susceptible to highly susceptible reaction against anthracnose of chilli. However, to draw a sound conclusion on the induction of resistance against anthracnose through Bion in the world, more and extensive studies are needed to elucidate the effect of Bion to induce resistance in chilli under field conditions for controlling anthracnose. Results of the present study may be suggestive rather than conclusive to take well planned research on the induction of resistance through Bion against anthracnose of chilli.

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