



Morpho-physiological Studies and Management Strategies of *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire Causing Dieback Disease of Chilli

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Abstract

India is one of the major chilli producing country in the world. Insect pest and diseases are one of the major constraints that attribute to low production and productivity in India. Dieback caused by *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire is one of the most important disease of chilli that affecting all the plant parts. *In vitro* effect of temperature pH and different nano compounds on the growth of *Alternaria tenuissima* showed that maximum mycelial growth (80.00 mm) was observed at 25°C while minimum mycelial growth (4.67 mm) at 10°C. Among different pH level, maximum mycelial growth 90.5 mm was recorded at pH 6.5 whereas minimum mycelial growth was recorded at pH 4.5. Maximum inhibition (71.92%) was recorded in silver nanoparticles @100 µg ml⁻¹ which was found at par with aluminium nanoparticles @ 100 µg ml⁻¹ (70.57%). Under field condition all the tested fungicides were found to be significantly superior over check in reducing incidence and severity of dieback disease of chilli. The minimum mean disease incidence and severity was recorded in Azoxystrobin 125 SC + Flutriafol 125 SC @ 14.28+14.28 g a.i ha⁻¹ 11.83% and 10.67% followed by Azoxystrobin 125 SC + Flutriafol 125 SC @ 12.5 + 12.5 g a.i.ha⁻¹ 14.92% and 14.0% over control respectively. The minimum mean fruit rot and maximum marketable yield were found in Azoxystrobin 125 SC + Flutriafol 125 SC @ 14.28+14.28 g a.i ha⁻¹ 30.54 q/ha and 25.51 q/ha followed by Azoxystrobin 125 SC + Flutriafol 125 SC @ 12.5 + 12.5 g a.i.ha⁻¹ 35.35q/ha and 24.61 q/ha respectively. The present studies concluded that Azoxystrobin 125 SC + Flutriafol 125 SC @ 14.28+14.28 g a.i ha⁻¹ could be used for the management of dieback disease and increase the yield of chilli.

Keywords

Chilli; *Alternaria tenuissima*; pH; Temperature; Nano compounds; Fungicides

Introduction

Chilli (*Capsicum annum* L.) is considered as one of the most important commercial spice crop and grown throughout the world for its green and red ripe fruits. India is one of the major chilli producing countries in the world. Chilli is cultivated over an area of 775 thousand hectares with an annual production of 1492 thousand tons and productivity of 1.9 metric tons per hectare in India [1]. Chilli fruit is used as fresh,

cooked, pickled and canned in sauces and as powder for hot spices. Pungency in chilli, which is due to the presence of capsaicin, is a digestive stimulant and a cure for rheumatic troubles. Among the spices consumed in India dried chilli powder contribute the major share. Green chillies are rich source of vitamins especially vitamin A, C, B1, B2 [2,3] and also rich in vitamin P (rutin), which is of immense pharmaceutical importance [4] and hence, it is recommended for the treatment of cholera, hoarseness, dropsy and colic toothache [5]

Chilli is affected by 750 pathogens of different origins, reported from different part of the world, but only few are responsible for considerable loss of production and productivity. Among the fungal diseases, dieback, leaf spot and fruit rot is caused by *Alternaria spp.*, damping off caused by *Pythium spp.*, *Phytophthora spp.* and other fungi, seedling blight caused by *Rhizoctonia spp.*, wilt caused by *Fusarium spp.*, anthracnose and dieback caused by *Colletotrichum capsici* are major diseases. Among all these *Alternaria spp.* are responsible for dieback, leaf spot and fruit rot have been identified as major limiting factor in chilli cultivation. The pathogens are seed, soil and air borne in nature [6-8].

This disease caused both pre- and post-harvest fruit decay [9]. It causes severe damage to leaves, twigs and fruits in the field as well as in storage and causes heavy loss up to 84 per cent [10]. Infected fruits lose their normal red colour and turn straw coloured or in some cases, pale white. Economic losses caused by the disease are mainly attributed to lower fruit quality and marketability.

Several conventional methods have been used for the control of these pathogens and each of these methods has one or other limitations. Some of these methods such as use of pesticides cause hazardous effect on the environment and human health. An understanding of the role of environmental conditions, temperature and pH effect on infection and survival of the pathogen is necessary to develop disease management practices. Use of nanoparticles has been considered an alternate and effective approach for the control of pathogenic microbes [11]. These nanoparticles have a great potential in the management of plant diseases as compared to synthetic fungicides [12]. Several workers have attempted to control dieback of chilli by use of different fungicides [13-16]. Considering the severity of this disease and its frequent occurrence in the fields, spoilage during transit and storage it has been felt necessity to develop effective management strategies. Thus, attempts have been made to determine the optimal conditions for the mycelial growth of the pathogen including temperature, pH and nano particles *in vitro* and also evaluate the efficacy of different fungicides against dieback of chilli for two consecutive years for the protection of disease with increase yield.

Materials and Methods

In vitro studies

Sampling and Isolation of *Alternaria tenuissima*: Dieback infected chilli leaves of Pant Chilli 1 variety were collected from Vegetable Research centre (VRC), Pantnagar, Uttarakhand in the months March and April. Leaves, twigs with spots were cut into small pieces, surface sterilized with 1% NaOCl for 1 minute rinsed thoroughly in sterile distilled water and dried on sterile filter paper. The leaf and

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twigs pieces were placed onto potato dextrose agar (PDA) medium and incubated at $25 \pm 20^\circ\text{C}$ for 07 days to promote fungal growth and sporulation. Pure cultures were obtained from single spores and hyphal tips. The morphological and culture characteristic of the isolated organism was studied, conidia and conidiophores details were considered. The identification was further confirmed from Indian Type Culture Collection (ITCC), IARI, New Delhi.

Effect of temperature on mycelial growth of *Alternaria tenuissima*: Six temperature regimes (5, 10, 15, 20, 25 and 30°C) were used to study the effect of temperature on mycelial growth of *Alternaria tenuissima* (Kunze ex Pers.) Wiltshire. Twenty millilitre of sterilized potato dextrose agar medium was poured in each sterilized Petri plate. Inoculation was made with 5 mm disc of 7 days old fungal culture in the centre of plate. Inoculated plates were incubated at $25 \pm 1^\circ\text{C}$ in BOD incubator. Observation on mycelial growth was recorded after 7th days of incubation. Each treatment was replicated five times.

Effect of different pH of media on the mycelia growth of *Alternaria alternata*: Eight pH levels (4.5, 5.0, 5.5, 6.0, 7.0, 7.5 and 8.0) were used to study the influence of pH on growth of *A. tenuissima* on PDA. Seven mm disc was cut from eight days old culture and placed in the centre of petridish and were incubated at 25°C . Colony characters were studied. Conidial pH was adjusted with the help of 0.1 NHCL and 0.1 N NaOH before sterilization. The radial growth of the fungal colony was assessed by measuring the growth of the colony diameter in mm. The observations were recorded after every 24 hrs for seven days in each experiment. The plates were incubated at temperature $25 \pm 20^\circ\text{C}$ for seven days.

Effect of nano compounds on mycelial growth of *Alternaria tenuissima*: To study the effect of nano compounds on the growth of the test pathogen *Alternaria tenuissima*, six nano compounds viz. Silver Nanoparticle, Aluminium Nanoparticle, Silicon carbide, Silicon dioxide, Titanium dioxide and Zinc oxide were tested at three concentrations (25, 50 and $100 \mu\text{gml}^{-1}$) [17]. Double strength of potato dextrose agar (PDA) was prepared and transferred in 150 ml flask at the rate of 50 ml per flask. A series of double concentration of each test nano compounds were prepared in 50 ml of sterile distilled water and added in each flask to get desired concentration, i.e. 25, 50 and $100 \mu\text{g ml}^{-1}$ of each nano compounds. Twenty milliliter of poisoned PDA was poured aseptically in each Petri plate and was inoculated with 5 mm mycelial disc. A suitable check was also maintained without adding any nano compounds in PDA. Each treatment was replicated five times and petri plates were incubated at $25 \pm 1^\circ\text{C}$ for 7 days. After 7 days of incubation, observations were recorded by measuring radial growth of the colony at right angles. Mean colony diameter was taken to calculate percent inhibition of radial growth by the formula given by Vincent [18].

$$\text{Percent growth inhibition} = \frac{\text{Colony diameter in check (-5 mm)} - \text{Colony diameter in treatment (-5 mm)}}{\text{Colony diameter in check (-5 mm)}} \times 100$$

In vivo studies

Experimental site: A field trial was carried out for two successive years during kharif seasons of the years 2014 and 2015 at the vegetable research centre of University, GBPUAT, Pantnagar in randomized block design with three replication. The Variety Pant chilli 1 was used for this study. Recommended agronomical package and practices were followed for all the treatments.

Fungicides used: To study the efficacy of different fungicides under field condition, ten different fungicides viz. chlorothalonil, propineb, azoxystrobin, krefoxym methyl, difenoconazole, tebuconazole,

hexaconazole, flutriafol, azoxystrobin + flutriafol and metiram + pyraclostrobin and control (without application of fungicide) were used against dieback of chilli. First spray was given just after the appearance of the disease symptoms followed by four more foliar sprays at 20 days interval. Fungicides were used as foliar spray and its dose is given in Table 1. Fungicide spray was made by knapsack sprayer of 15 liter capacity fitted with a hallow cone triple action nozzle. The 500 litre ha^{-1} water was used for each spray. The suspension of each fungicide was prepared separately by adding required quantity of respective fungicides in required amount of water. First spray was given just after the appearance of the disease symptoms followed by 4 more foliar sprays at 20 days interval.

Observations: After seven days of each spray observation on disease incidence and severity was recorded. Incidence of dieback was taken on plot basis by given formula.

$$\text{Percent fruit rot} = \frac{\text{Number of diseased fruits}}{\text{Total number of fruits}} \times 100$$

For disease severity five randomly selected plants were tagged in each plot and subsequent disease rating was done on scale of 0-5 suggested by Vishwakarma and Sitaramaiah [19] and Percent disease index (PDI) was calculated as described by McKiney [20].

$$\text{Percent fruit rot} = \frac{\text{Number of diseased fruits}}{\text{Total number of fruits}} \times 100$$

Harvesting was done at three different intervals on plot basis. Fruit yield was taken on plot basis and expressed as quintal/ha. Total numbers of healthy and infected fruits were recorded on five tagged plants per plot and percent fruit rot was calculated by following formula.

$$\text{Percent fruit rot} = \frac{\text{Number of diseased fruits}}{\text{Total number of fruits}} \times 100$$

Statistical analysis

The data was analysed statistically by Completely Randomized Design (CRD) or Randomized Block Design (RBD) using statistical software STPR developed by G. B. Pant University of Agriculture and Technology, Pantnagar. Data recorded were compared by the means of critical differences at one per cent level of significance in laboratory studies and five per cent level of significance in field studies.

Table 1: Details of fungicides used for the management of dieback disease of chilli under field conditions.

S.No.	Fungicide	Dosages	
		g a.i. ha^{-1}	Formulation g/ ml ha^{-1}
1	Chlorothalonil 75 WP	600	800
2	Propineb 70 WP	350	500
3	Azoxystrobin 25 SC	100	400
4	Azoxystrobin 25 SC	125	500
5	Kresoxym methyl 44.3 SC	250	500
6	Difenoconazole 25 EC	125	500
7	Tebuconazole 25.9 EC	125	500
8	Hexaconazole 2 SC	60	3000
9	Flutriafol 25 EC	100	400
10	Flutriafol 25 EC	125	500
11	Azoxystrobin 25 SC + Flutriafol 25 SC	12.5 + 12.5	800
12	Azoxystrobin 25 SC + Flutriafol 25 SC	14.28 + 14.28	914
13	Metiram 55 + Pyraclostrobin 5 WG	963 + 88	1750
14	Control	-	-

Result and Discussion

Effect of temperature on mycelial growth of *Alternaria tenuissima*

The fungus grew at most of the tested temperature (10, 15, 20, 25 and 30°C) except 5°C (Table 2). The entire tested temperature regime significantly influenced the growth of *Alternaria tenuissima*. Maximum mycelial growth (80.00 mm) was observed at 25°C while minimum mycelial growth (4.67 mm) at 10°C. At 30°C temperature growth was 70.00 mm followed by 41.33 mm and 13.67 mm at 20°C and 15°C, respectively. Results showed maximum mycelial growth of *Alternaria tenuissima* at 25°C and optimum growth at the temperature range 25-30°C. The results are in close conformity with the observations of Patil [21] who observed optimum temperature for growth and sporulation of *Alternaria tenuissima* lies from 25 to 30+2°C. Khare [22] and Milholland [23] reported 25 to 28°C optimum temperature for *Alternaria tenuissima*.

Effect of pH on mycelial growth of *Alternaria tenuissima*

The mycelial growth of *Alternaria tenuissima* was tested at eight different pH i.e. 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. At all the pH levels, the colony was fluffy type and pigmentation ranged from light brown to typical green black with concentric rings. The maximum mycelia growth 70.33 mm was recorded at pH 6.5 followed by 49.67 at pH 7.0 and 47.67 mm at pH 6.0. The minimum radial mycelia growth of the test pathogen was revealed at pH 4.5 (25.33 mm). This study showed that the maximum growth of this fungus at slightly acidic pH (Table 3).

Hydrogen ion concentration is the important factors that influence the growth and sporulation of the pathogen. Bhargava [24] and Mohanty [25] pointed out maximum colony growth and sporulation at pH 6.0 for *A. alternata*. Cakarevic and Boskovic [26] reported that the optimal pH value of fungus growth is between 6 and 8 depending upon the isolate. The results of the present study are in agreement with the conclusions made by Hubballi [27] who reported pH range 6.00-6.50 as optimum for growth of *A. alternata*.

Table 2: Effect of temperature on mycelial growth of *Alternaria tenuissima*.

S.No.	Temperature (°C)	Mycelial Growth (mm)
1	5	0.00
2	10	4.67
3	15	13.67
4	20	41.33
5	25	80.00
6	30	70.00
	CD at 1%	5.29
	CV	6.07

Table 3: Effect of pH on mycelial growth of *Alternaria tenuissima*.

S.No.	pH	Mycelial Growth (mm)
1	4.5	25.33
2	5.0	43.00
3	5.5	40.00
4	6.0	47.67
5	6.5	70.33
6	7.0	49.67
7	7.5	35.00
8	8.0	34.33
	CD at 1%	4.22
	CV	5.65

Effect of nanoparticles on mycelial growth of *Alternaria tenuissima*

In the present study, six nanoparticles viz. Silver Nanoparticles, Aluminium Nano Powder, Silicon carbide, Silicon dioxide, Titanium dioxide and Zinc oxide were evaluated at three different concentration i.e. 25, 50 and 100 µgml⁻¹ to record inhibition of radial growth of the test pathogen, *Alternaria tenuissima*. Data pertaining to colony diameter and per cent inhibition of radial growth presented in Table 4 revealed that all the nanoparticles significantly inhibited the growth of *A. tenuissima* as compared to control (Table 3).

At 25 µgml⁻¹ concentration mycelial growths varied from 25.00-73.0 mm. Minimum mycelial growth (25mm) was observed in Silver nanoparticles while maximum mycelial growth (73.00 mm) was observed in control. Silver nanoparticles gave highest per cent mycelial inhibition (65.75%) followed by aluminium nano powder (63.31%), titanium dioxide (53.88%), silicon carbide (48.24%) and silicon dioxide (46.60%). Zinc oxide was found least effective giving 43.58 per cent mycelial inhibition. Same trend followed at 50 and 100 µgml⁻¹ concentrations also upon increasing the concentration mycelial growth decreased significantly in all the nano compounds except titanium dioxide where no significant difference was observed between mycelial growth at 25 µgml⁻¹ and 50 µgml⁻¹ concentrations.

Several workers have been reported the fungi toxic action of silver nano particles against *Colletotrichum spp* [28], *Bipolaris sorokiniana* and *Magnaporthe grisea* [29], *Fusarium spp.* and *Phoma spp* [30]. Therefore nanoparticles like Silver, Aluminium, Titanium which has better fungicidal activity may be considered for devising the strategy for managing the diseases of chilli. Zinc oxide nanoparticles have also been observed to possess significant antifungal activity against *Fusarium sp.* in a concentration dependant manner [31]. Titanium dioxide was observed to have the maximum activity against *E. coli* and minimum activity against *Candida albicans* which was related to the complexity of the cell membrane [32]. Wani and Shah [33] reported antifungal activity of zinc oxide and magnesium oxide nanoparticles against *Alternaria alternata*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus*.

Above studies support the findings of the present study. Thus it can be suggested that silver and aluminium nanoparticles can be included in integrated pest management programmes because these nano compounds found to inhibit the fungal growth.

Effect of different fungicides on incidence of dieback disease of chilli

The effect of different fungicides revealed that all the fungicides reduced the disease incidence significantly as compared to check in kharif 2014 and 2015 (Figure 1). Maximum disease incidence (46.67% and 58.89%) was recorded in check and minimum in Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ (11.43% and 12.22%) which was at par with Azoxystrobin 125 SC + Flutriafol 125 SC @ 12.5 + 12.5 g a.i.ha⁻¹ (14.29% and 15.56%) in both kharif 2014 and 2015. The minimum mean percent disease incidence of both the seasons was found in Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ followed by Azoxystrobin 125 SC + Flutriafol 125 SC @ 12.5 + 12.5 g a.i.ha⁻¹ (Figure 2).

Thus fungicides used for managing the chilli diseases play important role towards the management of chilli diseases. This result also confirmed the work of Mazur [34] who found that azoxystrobin was found effective in controlling the diseases caused by *Alternaria*

Table 4: Effect of Nano particles on mycelial growth of *Alternaria tenuissima*.

Treatment	Mycelial growth (mm)			Growth Inhibition (%)		
	25 µg ml ⁻¹	50 µg ml ⁻¹	100 µg ml ⁻¹	25 µg ml ⁻¹	50 µg ml ⁻¹	100 µg ml ⁻¹
Silver Nanoparticles	25.00	23.82	21.48	65.75	67.37	70.57
Aluminium Nano Powder	26.78	23.00	20.50	63.31	68.49	71.92
Silicon carbide	37.78	33.97	32.48	48.24	53.47	55.50
Silicon dioxide	38.98	37.65	36.50	46.60	48.42	50.00
Titanium dioxide	33.67	33.48	32.48	53.88	54.13	55.50
Zinc oxide	41.18	38.10	35.00	43.58	47.81	52.05
Control	73.00	73.00	73.00	-	-	-
CD at 1% Fungicide Concentration		0.77				
Fungicide x Concentration		0.50				
CV		1.33				
		2.14				

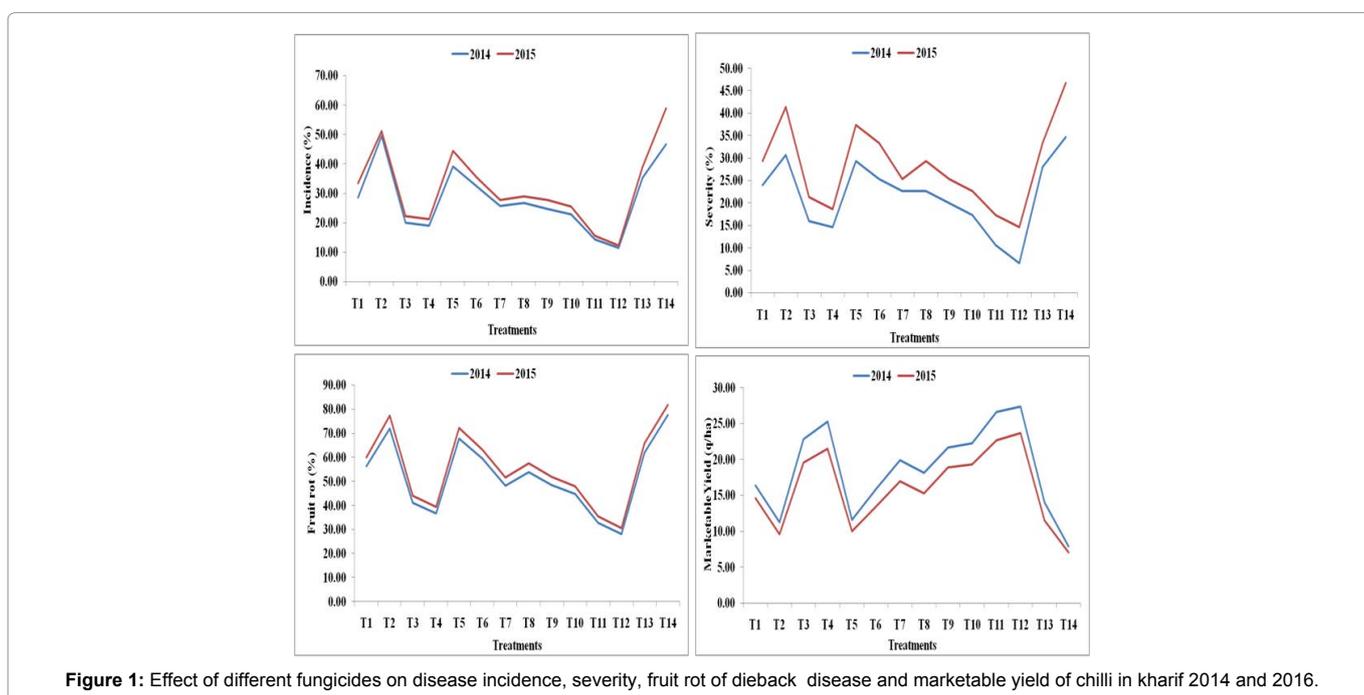


Figure 1: Effect of different fungicides on disease incidence, severity, fruit rot of dieback disease and marketable yield of chilli in kharif 2014 and 2016.

tenuissima. Fugro [35] recorded that the fungicidal sprays of chlorothalonil (0.25%) reduced the incidence of *Alternaria* blight of tomato by 20 per cent. Therefore Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ which have better control over disease can be used for chemical control of chilli.

Effect of different fungicides on severity of dieback disease of Chilli

The Maximum and minimum disease severity in kharif 2014 and 2015 were recorded in Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ and check i.e. 6.67%, 14.67%, 34.67% and 46.67% respectively (Figure 1). The minimum mean disease severity of both the season was found in Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ (10.67%) followed by Azoxystrobin 125 SC + Flutriafol 125 SC @ 12.5 + 12.5 g a.i.ha⁻¹ (14.00%) in comparison to check (40.67%) (Figure 2).

Bal [36] also observed that propineb gave excellent control against *Alternaria* leaf blight of strawberries incited by *A. tenuissima*. Mazur [34] who found that azoxystrobin was found effective in controlling the diseases caused by *Alternaria tenuissima*. Fugro [35] recorded

that the fungicidal sprays of chlorothalonil (0.25%) reduced the incidence of *Alternaria* blight of tomato by 20 per cent. Efath [37] who reported that hexaconazole at 0.06% was most effective in managing foliar blight of onion caused by *A. tenuissima*. The present study showed that Azoxystrobin 125 SC + Flutriafol 125 SC, Azoxystrobin 23 SC and Flutriafol 250 EC can be used for chemical control of chilli towards the management of leaf spot, fruit and early dieback of chilli.

Effect of different fungicides on fruit rot of chilli

The effect of different fungicides revealed that fruit rot of chilli reduced in all the treatments as compared to check in both kharif 2014 and 2015 (Figure 1). The maximum mean fruit rot of chilli was recorded in check (79.63%) while minimum fruit infection was recorded in Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ (29.32%) followed by Azoxystrobin 125 SC + Flutriafol 125 SC @ 12.5 + 12.5 g a.i.ha⁻¹ (34.10%), Azoxystrobin 23 SC @ 125 g a.i. ha⁻¹ (37.99%), Azoxystrobin 23 SC @ 100 g a.i. ha⁻¹ (42.52%) (Figure 2). The results of the present study are in accordance with Raja [38] who found hexaconazole highly effective against *A. tenuissima*. Naik and Sabalpara [39] also observed that fungicides viz. difenoconazole 1ml/l hexaconazole 1ml/l, chlorothalonil 1.5g/l, propineb 2.5 g/l, gave

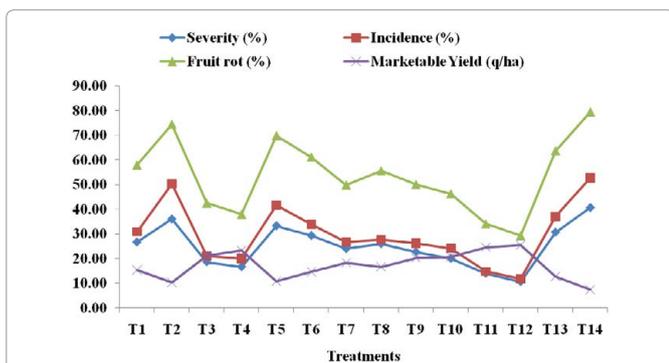


Figure 2: Effect of different fungicides on mean disease incidence, severity, fruit rot of dieback disease and marketable yield of chilli.

high disease control against *A. tenuissimia*. Azoxystrobin 125 SC + Flutriafol 125 SC, Azoxystrobin 23 SC and Flutriafol 250 EC were tested for the first time on chilli in present study against fruit rot of chilli.

Effect of different fungicides on fruit yield of chilli

The results of the experiment presented in Figures 1 and 2 showed that all the treatments increased the yield significantly as compared to check in both the kharif season. In kharif 2014 and 2014, the lowest marketable yield (7.89 q/ha and 7.06 q/ha) were recorded in check whereas highest marketable yield (27.33 q/ha and 23.69 q/ha) were found in Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ which was at par with Azoxystrobin 125 SC + Flutriafol 125 SC @ 12.5 + 12.5 g a.i.ha⁻¹ (26.55q/ha and 22.66) respectively. The maximum and minimum mean fruit yield showed in treatment Azoxystrobin 125 SC + Flutriafol 125 SC @ (14.28+14.28) g a.i ha⁻¹ and check respectively.

The results are in accordance with the studies of Kushwaha [40] who reported that fungicide application increased the yield. Similarly, Mohan [41] who reported that the triazole fungicides gave highest pathogen inhibition and produced highest yields. Devanathan and Ramanujam [42] reported that foliar spray of Chlorothalonil (0.2%) reduced the disease intensity and increased yield significantly as compared to unsprayed plot in case of early blight of tomato Azoxystrobin 23 SC, Flutriafol 250 EC and Azoxystrobin 125 SC + Flutriafol 125 SC have not been tested earlier.

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