Research Article

Disease intensity and eco-friendly management of *Alternaria alternata* in chili (*Capsicum annuum* L.)

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Abstract

Fruit rot of chili caused by *Alternaria alternata* is the most commonly occurring fungi affecting the crop. Present study was carried out on survey, intensity and management of causal fungus of chili fruit rot. The chili fruit rot incidence during the survey of fields/commercial markets carried out in the surroundings of Hyderabad, Mirpur Khas, Tando Allahyar and Umer Kote districts was ranged from 14.33-36.66 %. On overall basis Umer Kote district revealed significantly highest (31.44%) fruit rot incidence compared to Tando Allahyar (26.89%) and Hyderabad (26.56%). However, it was lowest in Mirpur Khas (19.00%). Among the 12 different locations (villages and markets) from four major chili growing districts, maximum incidence of chilli fruit rot was recorded at Sultanabad (36.66%) followed by Village Mustafa Arain (35.33%), whereas, the minimum fruit rot incidence (14.33%) was observed at Khawaja Village followed by Dah hoti (18.66%). All the 22 tested botanical treatments gave inhibitory effects; however, significantly lowest radial mycelial colony growth and greatest reduction of *A. alternata* was induced by extract from *Amomum subulatum* (22.94mm and 74.51%) followed by *Azadirachta indica* seed (24.15mm and 73.16%). The maximum radial mycelial colony growth and lowest reduction of *A. alternata* was recorded in control (90.00mm and 00.0%) followed by the extract from *Syzygium aromaticum* (66.17mm and 26.48%). The efficacy of other tested botanicals remained moderate. Hence several botanical extracts which are more effective in suppressing chilli fruit diseases caused by *A. alternata* may be commercially prepared and used for integrated chilli fruit rot management, with a hope that it will be helpful for the end users.

Key words: *Amomum subulatum*; Botanical extracts; Chili fruit rot; Fungus

Introduction

Chilli (*Capsicum annuum* L.) is believed to be one of most important tropical and subtropical crops grown worldwide due to its high nutritional value, consumption in

daily life and cash value to growers and consumers [1]. It belongs to the genus *Capsicum* and Solanaceae family [2] and commonly called as red pepper. Amazingly high levels of vitamins and minerals are

available in chilli hot peppers [3]. Generally red chilli peppers are processed in to chilli powder or paprika, depending on the pungency of peppers. The spice has numerous uses for commercial, nutritional as well as medicinal [4]. Internationally, chillies are one of the biggest imported and exported spices of the world. Pakistan is among the biggest producer of chillies after China, India, and Mexico. It is one of the major crops of Pakistan due to high quality production and important ingredient in daily diet. It is reported that during 2014-15 chillies and peppers (green and dry) were harvested about 1688082 and 1937370 hectares in the world, with a production of 3818768 and 32324345 tonnes, respectively. However, the area harvested for chillies and peppers (dry) in Pakistan was 62742 hectors, producing 145856 tonnes during 2014-15 [5]. In addition, it is considered an important cash crop of the Sindh province, particularly in the taluka Kunri of District Umer Kot, which is considered as major belt of chilli production in Asia. Recently, this area was known as the chillies capital of the world [6].

Several losses in both quality and quantity have been faced that causes decline in yield, however, major problems includes improper management, insect pests and diseases during pre and post-harvest stages etc. [7]. Several pathogenic fungi may be involved in causing diseases in chilli [8]. In the literature, there are several evidance indicating the association of fungal species with chilli such are *Alternaria alternata*, *F. anthophilum*, *F. oxysporum*, *F. proliferatum*, *F. moniliforme*, *F. solani*, *Cephalosporium acremonium*, *M. phaseolina*, *Pythium aphanidermatum* and *R. solani* [8]. However, amongst all above, fruit rot caused by *A. alternata* is the most commonly occurring fungi in the harvested fruit of chilli. The disease was first reported in 1950s in New Mexico as an internal mold of red peppers, most noticeable after frost [9]. Symptoms of *Alternaria* rot began as gray and water-soaked lesion on either the

slide of blossom-end of the fruit [10]. As the lesion progress, they darken and become covered the spores. Internal necrosis and mycelial growth occurs on the seeds, placenta and pericarps [11].

Leaf spot disease caused by *Alternaria alternata* (Fr) Keissler is becoming a limiting factor and posing a major problem in chilli production [12, 13]. Several management practices have been used to cater this major problem in chilli, however, due to some concerns are not recommended for large scale. Managing the chilli fruit rot disease exclusively by using chemical pesticides being undesirable due to health hazards and associated risks of environmental pollution. Thus, there is dire need to go for biorational alternatives that are eco-friendly. Various plant based products and extracts are encouraged due to no health hazard and environmental pollution [14]. The mycelial growth of *A. tenuis* was inhibited by the use of garlic bulb extract [15]. The highest efficacy against *A. brassicicola* for ethanol extract of *Polygonum perfoliatum* (speed weed) was reported in another study [16]. Other methods of managing *Alternaria* pathogens like use of bio-control agents, medicinal plants, various plant products etc. have some potential. All above mentioned studies reading the management has been used against different species of *Alternaria*, however, a very limited work is reported for *Alternaria alternata* (Fries) Keissler especially with reference to non-chemical measure for the management. Thus, present study was conducted to estimate the intensity and possible eco-friendly management of *Alternaria alternata* by investing the in vitro assessments of different botanicals.

Materials and methods

Estimation of diseases intensity of fruit rots of chilli

Survey for fruit rot disease in major chilli growing districts of Sindh viz; Hyderabad, Tando Allahyar, Mirpur Khas and Umer Kote (Fig. 1) was conducted in order to estimate disease intensity. A total of three

villages and/or commercial markets were selected from each districts as Hyderabad (Kasana Mori, Village Dur M. Pathan, Village Mustafa Arain), Mirpurkhas (Village Iqbal Chaudheri, Khawaja Stop, Village Khaaia), Tando Allahyar (Sultanabad, Tand Allahyar bypass, Dah hoti) and Umer Kote (Kunri Market, Village Jamali, Village Mured Pousio). Three spots (replications) per location were

randomly observed for disease intensity percentage. 100 fruits from ten different plants were randomly observed based on the visual symptoms produced by fruit rot causing fungi. Similarly, 100 fruits from commercial market were also randomly collected for observation. The disease intensity was calculated by using the formula of diseases incidence as under:

$$\text{Diseases Incidence (\%)} = \frac{\text{Number of Infected fruits}}{\text{Total Number of Observed Fruits}} \times 100$$

Isolation and identification of fungus causing chilli fruit rot

The samples of chilli fruit showing typical symptoms of fruit rot were collected and processed for the isolation of the fungus. The collected samples were thoroughly washed with tap water. Small pieces of infected portion about 2-3mm in length were cut at the junction of diseased and healthy tissues with the help of alcohol sterilized sharp blade. These pieces were surface sterilized in 0.1 per cent mercuric chloride solution (HgCl₂) for 30 seconds followed by three washing with sterilized distilled water in beakers under aseptic conditions using laminar air flow. The pieces were then

completely dried by placing on sterilized blotting paper. Five bits were transferred aseptically to the petriplates containing sterile potato dextrose agar (PDA) medium amended with an antibacterial agent and filled up to quarter strength. The inoculated plates were incubated at 25°C. All the plates were monitored regularly and growing colonies were subjected to different laboratory codes for frequency percentage and further analysis. About 30-35 isolations were made throughout the experiment. The frequency of the fungi in the collected specimens from each locality was recorded by using the following formula:

$$\text{Frequency (\%)} = \frac{\text{Number of Pieces colonized}}{\text{Total Number of Pieces studied}} \times 100$$

The culture, thus, obtained was subjected to purification. A single spore culture technique was used to purify the isolates. Sub-culturing of isolates were made time to time to maintain the fresh culture for further analysis until the end of experiments. Temporary slides of fungal isolates from pure cultures were made and observed under light microscope. Morphological and cultural characters of isolated fungi were recorded and compared with standard keys for establishing their identity [17].

In vitro evaluation of botanicals against *Alternaria alternata*

A total of 22 different botanicals extracts were assessed in vitro condition against the fungus causing fruit rot of chilli. Poison bait

technique was followed to assess the antifungal potential of different botanical extracts [18]. The basal medium was amended with 5 % autoclaved aqueous extracts of different botanical. The experiments were laid out in complete randomized design (CRD) with six replications. Petri plates contained amended PDA medium with different botanicals were inoculated with 5 mm disk of freshly prepared culture of fruit rot causing fungus under controlled conditions. The un-amended PDA plates were kept as control. All the treatment was incubated at 25±1°C. The data on radial mycelial growth in both treated and control was recorded on daily basis until the colony growth of

control plates became full. The percent inhibition of radial mycelial growth over control was calculated by using the formula suggested by [19] $I = (C-T)/C \times 100$, Where,

I = Per cent inhibition, C = Radial colony growth in control, T = Radial colony growth in treatment.

Figure 1. Map showing the major chili growing districts of Sindh for the estimation of diseases intensity of fruit rots

Results

Fruit rots intensity in major chilli growing districts of Sindh

The incidence of chilli fruit rot in three different locations (villages and markets) of each districts indicate the significant ($P < 0.05 = 0.0092$) difference. Similarly, significant difference ($P < 0.05 = 0.0001$) was noticed on overall basis among all four observed districts. Maximum incidence chilli fruit rot (36.66%) was recorded at Sultanabad followed by Village Murad Pousio (35.33%), Jamali village (33.66%), Dur Muhammad Pathan (32.66%).

Whereas, the minimum fruit rot incidence (14.33%) was observed at Khawaja Village followed by Dah hoti (18.66%), Village Iqbal Chaudheri (20.66%) (Fig. 2A).

The overall fruit incidence of all four observed districts revealed the highest incidence (31.44%) in Umer Kote followed by Tando Allahyar (26.89%) and Hyderabad (26.56%), however, no significant difference was noticed for Tando Allahyar and Hyderabad. Whereas, the lowest fruit rot incidence was observed in Mirpur Khas (19.00%) (Fig. 2B).

Figure 2. (A) Diseases incidence percentage recorded from different villages and commercial markets of major chilli growing districts of Sindh, Pakistan. (B) Overall diseases incidence percentage recorded from major chilli growing districts of Sindh, Pakistan. (D1: Hyderabad, D2: Mirpurkhas, D3: Tando Allahyar, D4: Umer Kote)

Morphological characteristics of *Alternaria alternata* associated with fruit of chilli

The *A. alternata* was indentified based on the morphological characteristics. The purified culture of the *A. alternata* on PDA produced aerial mycelium, initially greenish white in colour later changed to greyish black with black reverse. Microscopic examination revealed septate brown hyphae, with septate and brown

conidiophores bearing conidia in chains. Conidiophores were pale brown, simple and branched, bearing catenulate conidia at the apex and apical fertile parts. Conidia catenulate, mostly up to 9 in a chain, often branched. Conidia were prosperous, acropetally developed, dark brown, cylindrical or spindle-shaped, often with cylindrical beaks, muriform composed of 3–4 transverse walls and 1–2 longitudinal walls (Fig. 3).

Figure 3. Morphological characteristic of *Alternaria alternata* isolated from chilli fruit rot (A: Radial colony growth; B: Acropetally developed chain of conidia; C: Immature conidia; D: Mature conidia of *Alternaria alternata*)

In vitro* antifungal potential of different botanical against *A. alternata

The *in vitro* antifungal potential of 22 different plants extracts used by poison bait techniques in petri dishes revealed varied responses to reduce the *A. alternata* radial mycelial colony growth, and showed fungicidal effects for chilli fruit rot. All the tested botanical treatments gave inhibitory effects. However, the minimum radial mycelial colony growth of *A. alternata* was observed by extract from *Amomum subulatum* (22.94mm) followed by *Azadirachta indica* seed (24.15mm), *Azadirachta indica* leaves (27.64mm), *Piper nigrum* (White) (35.12mm) and *Allium sativum* (36.52mm) compared to control (90.00mm). No significant difference was observed between *Amomum subulatum* and *Azadirachta indica* seed. Similarly, no significant difference was observed between *Piper nigrum* (White) and *Allium sativum*. The maximum radial mycelial colony growth of *A. alternata* was recorded in control (90.00mm) followed by the extract from *Syzygium aromaticum* (66.17mm), *Coriandrum sativum* (65.83mm), *Eucalyptus grandis* (65.25mm) and *Cinnamomum tamala* (61.50mm) (Fig. 4A, C).

The efficacy of all the tested botanical extracts gave inhibitory effects. The significantly greatest reduction of radial mycelial colony growth of *A. alternata* was induced by extract from *Amomum subulatum* (74.51%) followed by *Azadirachta indica* seed (73.16%) and *Azadirachta indica* leaves (69.28%) While the lowest reduction of radial mycelial colony growth of *A. alternata* was induced by extract from *Syzygium aromaticum* (26.48%) followed by *Coriandrum sativum* (26.85%), *Eucalyptus grandis* (27.50%) and *Cinnamomum tamala* (31.667%) (Fig. 4B).

Discussion

Present investigation was planned to conduct the survey, intensity and possible management of fungus causing fruit rot of chili using non-chemical measures,

particularly botanicals against fruit causing fungus under *in vitro* conditions. Considering all these points and importance of crop, the present investigation was taken up to generate the information regarding the fruit rot intensity and *in vitro* management with different botanical extracts. Total of 22 different botanical extracts were used to test the antifungal potential against *Alternaria alternata*.

In nature chilli fruit rot affecting the crop from early stage of fruiting to maturity and continue till harvest; thus, considered as a serious threat to chilli crop [20, 21]. In the present study chilli fields/commercial markets surveyed in the surroundings of Umer Kote, Mirpur Khas, Tando Allahyar and Hyderabad, during the month of February and March, 2017, where chilli fruit rot incidence was ranged from 14.33 to 36.66 percents. On overall basis Umer Kote district revealed the highest fruit rot incidence compared to Tando Allahyar and Hyderabad. It was lowest in Mirpur Khas that maybe due to variability in the climatic conditions over there. The incidence of chilli fruit rot recorded from 12 different locations (villages and commercial markets) of four observed districts showed great variability that maybe due to environmental conditions, management practices followed and quality of seeds as well. However, in other studies recently conducted reported overall 60.33 per cent infected fruit during survey of four locations of Jaipur district in 2015 and it was ranged from 51.75-66.70% [22]. Therefore, fruit rot is a major threat to chilli in Jaipur district that cause profitable loss in yield. Mean disease incidence and disease intensity of *Alternaria alternata* *ceratoni* blight in adjoining areas of El-Beida City of Libya ranged from 54-96% and 14-43.6%, respectively have also been reported [23]. Though, their incidence percent compared to current study was higher, but that maybe due to environmental factors and varietal response.

Figure 4. (A) Efficacy percentage of different botanical extracts against *A. alternata*. (B) Radial mycelial colony growth of *A. alternata* affected by different botanical extracts (C) In vitro performance of different botanical extracts against *A. alternata*.

T1(leaves; *Azadirachta indica* L.), T2(Seed; *Azadirachta indica* L.), T3(*Nicotiana rustica* L.), T4(*Caloptropis gigantean*), T5(*Eucalyptus grandis*), T6(*Ocimum basilicum*), T7(*Ficus benghalensis*), T8(*Mentha spicata* L.), T9(*Cuminum cyminum*), T10(*Coriandrum sativum*), T11(*Piper nigrum*), T12(*Capsicum annuum* L.), T13(*Curcuma longa*), T14(*Syzygium aromaticum*), T15(*Cinnamomum tamala* L.), T16(*Allium sativum*), T17(*Allium cepa*), T18(*Cinnamomum Zeylanicum* L.), T19(*Zingiber officinale*), T20(*Illicium verum*), T21(*Amomum subulatum*), T22(*Piper nigrum*), C (Control).

In the current study, the identification of fungi revealed that *Alternaria alternata* (Fr.) Keissler based on the morphological characteristics was established as main cause of fruit rot and has been

predominantly observed. It produced aerial mycelium, initially greenish to white in colour then changed to greyish black with black reverse on PDA medium. Conidia catenulate, mostly up to 9 in a chain, often

branched. Conidia were acropetally developed, dark brown, cylindrical or spindle-shaped and composed of 3–4 transverse walls and 1–2 longitudinal walls. The results of current study were in confirmation with the results of various other workers [21, 24].

In the recent study it was revealed that *Colletotrichum capsici*, *Fusarium moniliforme*, *F. pallidroseum*, *F. oxysporum*, *Alternaria alternata* and *Aspergillus flavus* were found associated with the chilli fruit rot [21]. In addition there are several other studies reported *A. alternata* as causal organism of chilli fruit rot [25]. It was also found *A. alternata*, *F. moniliforme* and *C. capsici* pathogenic on chilli fruit [25]. In another work researchers mentioned *A. alternata*, *C. capsici*, *F. moniliforme*, *F. oxysporum* and *F. pallidroseum* as the causal organism of different fruit rots of chilli [27].

Managing the chilli fruit rot disease exclusively by using chemical pesticides being undesirable due to health hazards and associated risks of environmental pollution. Thus, there is a dire need to go for biorational alternatives that eco-friendly. It is obvious that integrated or eco-friendly disease management has holds considerable importance in all crops and especially in vegetable crops is more worthwhile due to its consumption in various ways. Keeping all these and importance of chilli, the present study was carried out use some botanical pesticides as alternative against *A. alternata*, the causal organism of chilli fruit rot. Total of 22 different plants extracts were used by poison bait techniques in the current study in order to test *in vitro* antifungal potential against *A. alternata* radial mycelial colony growth. The results revealed the fungicidal effects of different botanicals for chilli fruit rot causing fungi. All the tested botanical treatments gave inhibitory effects; however, significantly greatest reduction of radial mycelial colony growth of *A. alternata* was induced by extract from Black Cardamom Pods followed by Neem seed, Neem leaves,

White pepper and Garlic compared to control. However, no significant difference was observed for antifungal efficacy between *Amomum subulatum* and *Azadirachta indica*. The lowest reduction of radial mycelial colony growth of *A. alternata* was induced by extracts from *Syzygium aromaticum* followed by *Coriandrum sativum*, *Eucalyptus* and *Cinnamomum tamala*. The present investigation used eco-friendly management and evaluated different non-chemical measures, particularly botanicals pesticides for possible management of fruit causing fungus under *in vitro* conditions. In other studies, for the management of chilli fruit rot, conventional production system was used to manage *Alternaria* pathogens that are very difficult. They used chemical fungicides and got satisfactory control but are dangerous to the ecosystem [28]. However, resistant germplasm were evaluated against chilli fruit rot causing pathogens that is also time consuming [21]. The use of plant based products and extracts are encouraged due to no health hazard and environmental pollution. Similar to current study, the extracts of *Canna indica*, *Convolvulus arvensis*, *Ipomoea palmata*, *Cenchrus catharticus*, *Mentha piperita*, *Prosopis spicigera*, *Allium cepa*, *A. sativum*, *Lawsonia inermis*, *Argemone mexicana*, *Datura stramonium* and *Clerodendron inerme* were used against *A. brassicae* [14]. The mycelial growth of *A. tenuis* was inhibited by the use of *Allium sativum* bulb extract have been reported by [15]. The strong efficacy of ethanol or methanol extract of speed weed (*Polygonum perfoliatum*) against *A. brassicicola* was reported [16]. The *Azadirachta indica* leaf extract showed significant reduction of radial colony growth of *A. solani* [2]. Other methods of managing *Alternaria* pathogens like use of bio-control agents, medicinal plants, various plant products etc. have some potential. All above mentioned studies reading the management has been used are inconsistent with current duty because they

used different species of *Alternaria*; however, some plant based extracts such as neem leave, garlic, onion and mint were used by other researchers [14, 15, 29]. The current studies used 22 different plant based bio-pesticides against *Alternaria alternata* (Fries) Keissler as an alternative non-chemical measure for the eco-friendly and sustainable chilli fruit rot management. Hence it has been proved that there are several botanical extracts which are more effective in suppressing chilli fruit diseases caused by *Alternaria alternata* (Fries) Keissler without environmental pollution and health hazards. The results of current finding revealed that plant based biopesticides are environmentally friendly and cost effective maybe used for integrated chilli fruit rot management with hope that it will be helpful for the end users.

Conclusion

Chili fruit rot caused by *A. alternata* commonly found in districts of Sindh Province. Several botanicals were found to be active against *A. alternata* in-vitro. These botanicals can be used for the eco-friendly management of this disease in order to minimize the losses.

Authors' contributions

Conceived and designed the experiments: HU Soomro & MI Khaskheli, Performed the experiments: HU Soomro, M Hyder & AA Bukero, Analyzed the data: A Bukero, S Panhwar & RAA Khan, Contributed materials/ analysis/ tools: MI Khaskheli, Wrote the paper: RAA Khan, HU Soomro & AQ Larik.

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