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Full Length Article

Effect of Incorporation of Leaf Biomass of *Coronopus didymus* on Management of Basal Rot Disease of Onion and its Physiology

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Abstract

Fusarium oxysporum f. sp. *cepae* is a soil-borne fungal pathogen that causes basal rot disease of onion (*Allium cepa* L.) resulting in significant yield losses of the crop. The present study was conducted to assess the disease management potential of *Coronopus didymus* (L.) Sm., and its effect on onion plant physiology. In laboratory bioassays, different concentrations ranging from 1% to 7% of methanolic extracts of leaves, stems and roots of *C. didymus* reduced fungal biomass by 60–84%, 51–85% and 73–87%, respectively. In a pot trial, dry leaf biomass of *C. didymus* was mixed in the soil at 1%, 2% and 3% in combination with *F. oxysporum* f. sp. *cepae* and their effect on disease development, plant growth and various physiological parameters was studied in comparison with a negative control (without any addition of pathogen or plant material) and a positive control inoculated only with the fungus. The highest disease incidence (47%) was recorded in positive control. Application of 2% and 3% dry leaf biomass of *C. didymus* as soil amendment significantly reduced disease incidence to 13% and 3%, respectively. Similarly, plant mortality was reduced to 0% due to 2% as well 3% dry leaf biomass treatments. The highest chlorophyll, sugar and protein content were recorded in treatment where *F. oxysporum* f. sp. *cepae* was inoculated with 1% dry leaf biomass incorporation. On the other hand, the highest phenolic content and peroxidase activity were recorded due to combined application of the pathogen and 3% dry biomass incorporation. Present study concludes that soil amendment with 2% leaf dry biomass of *C. didymus* can effectively manage the basal rot of onion without any adverse effect on plant growth.

Keywords: Basal rot of onion; *Coronopus didymus; Fusarium oxysporum* f. sp. *cepae;* Plant physiology

Introduction

Onion is among the most important vegetables of Pakistan and many parts of the world. It has nutritional importance and contains sufficient quantities of flavonoids namely quercetin and anthocyanin, which are beneficial for health. Compounds present in onion have a number of health benefits including anticarcinogenic properties, antibiotic effects as well as antithrombotic, antiasthmatic and antiplatelet activities (Griffiths *et al.,* 2002). Onions are attacked by several root, bulb and foliar fungal pathogens that decrease quality and yield of the crop. Among these, *F. oxysporum* f. sp. *cepae* is a highly important soil-borne fungal pathogen that causes basal plate rot disease in onion worldwide (Cramer, 2000; Taylor *et al.,* 2012). The fungus causes infection in roots or the basal plate of onion bulbs. Later in the season, infection spreads in onion bulb scales. However, severe losses occur during storage (Rabiei-Motlagh *et al.,* 2010). Apart from onion, *F. oxysporum* f. sp. *cepae* also attacks other *Allium* species namely Welsh onion (*A. fistulosum* L.), shallots (*A. cepa* var. *ascalonicum*), garlic (*A. sativum* L.) and chives (*A. schoenoprasum* L.) (Havey, 1995).

Although *F. oxysporum* f. sp. *cepae* can produce macroconidia and microconidia, however, in the field chlamydospores are major form of the pathogen inoculum (Cramer, 2000). The clamydospores live long in the soil and are difficult to eradicate (Brayford, 1996). The disease is generally managed by crop rotation and cultivation of resistant varieties (Havey, 1995; Ozer *et al.,* 2004; Taylor *et al.,* 2012). However, generally partial resistance has been found in onion against basal rot pathogen (Cramer, 2000). Various chemical methods have been reported for the control of *F. oxysporum* f. sp. *cepae*. Soil fumigation with metham sodium or methyl bromide has been reported to be successful for control of the pathogen (Jaworski *et al.,* 1978; Nico and Sánchez, 2012). Treatment of seeds, sets or transplants with certain fungicides such as benomyl, tebuconazole, thiram and prochloraz can reduce the fungal infection (Koycu and Ozer, 1997; Ozer and Koycu, 1998; Taylor *et al.,* 2012). However, chemical fungicides also have ill effects on environment as well as human's and animal's health. Scientists are, therefore, in search of environment friendly alternatives to these chemical

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fungicides for the management of plant pathogens. In the recent years, various studies have revealed that crude extracts as well as purified compounds of plants have a great potential in the management of plant pathogens (Kanwal *et al.,* 2010; Jabeen *et al.,* 2011; Javaid and Khan, 2016; Khurshid *et al.,* 2016). Previous studies have shown that soil amendments with plant materials of members of family Brassicaceae significantly reduced disease incidence caused by fungal pathogens (Subbarao *et al.,* 1994). However, generally these studies were carried out using cultivated plants of Brassicaceae and studies regarding the use of Brassicaceous weeds are scarce. The objectives of the present study were, (i) to investigate the antifungal potential of methanolic extracts of different parts of a brassicaceous weeds *Coronopus didymus* against *F. oxysporum* f. sp. *cepae*, and (ii) to study the effect of soil amendment with different doses of dry biomass of *C. didymus* on management of basal rot disease of onion and various plant physiological parameters.

Materials and Methods

Isolation of *Fusarium oxysporum* f. sp. *cepae*

Samples of onion bulbs infected with basal rot disease were collected from a field of Mandi Bahaud-Din, Pakistan. Surface sterilization of the infected parts was accomplished with 1% solution of sodium hypochlorite for 1 minute. These pieces were transferred to potato dextrose agar in Petri plates and incubated for 7 days at 23ºC in the dark. The isolated fungus was sub-cultured using a single spore technique and purified culture was identified as *F. oxysporum* f. sp. *cepa* with reference to the literature (Leslie and Summerell, 2006).

Screening Bioassays

Leaves, stems and roots of *C. didymus* were collected from Lahore, Pakistan and dried under sunlight. The plant materials were thoroughly crushed and stored in polythene bags. Two hundred grams of each crushed plant materials were soaked in 1.0 L methanol separately for 2 weeks. After that the soaked material was filtered through muslin cloth followed by filter paper. The filtrates were evaporated on rotary evaporator at 45°C to get crude methanolic extracts of different parts. Crude extract (22.4 g) of each plant part was dissolved in 7 mL dimethyl sulphoxide (DMSO) and final volume (28 mL) was raised by autoclaved distilled water to prepare stock solution. A control solution was also prepared by adding 7 mL DMSO to 21 mL of distilled water. Seventy three milliliter malt extract was autoclaved in 250 mL flasks and cooled down. Seven concentrations viz 1, 2, 3, 4, 5, 6 and 7% were prepared by adding, 1, 2, 3, 4, 5, 6 and 7 mL of stock solution with 6, 5, 4, 3, 2, 1 and 0 mL of control solution, respectively, to raise the volume of the medium to 80 mL in each flask. The 80 mL of each treatment was divided into four equal portions in 100 mL flasks to serve as replicates. Control treatment was also prepared by adding 7 mL control solution to 73 mL autoclaved malt extract broth. Mycelial discs of *F. oxysporum* f. sp. *cepae* were prepared from the tips of 7 days old fungal culture using a sterilized 5 mm diameter cork borer and transferred to each flask. Flasks were incubated at 27±1°C for 7 days. After 7 days the fungal biomass in each flask was filtered, dried in an electric oven at 60°C and weighed on an electric balance (Javaid and Akhtar, 2015).

Pot Trial

Pot trial was carried out by making the pot soil inoculating with *F. oxysporum* f. sp. *cepae* and amending the soil with dried leaves of *C. didymus.* For preparation of inoculum, 0.5 kg of chickpeas were boiled and autoclaved at 121°C for 30 minutes in plastic bags. After autoclaving, these were cooled at room temperature and inoculated with fresh culture of *F. oxysporum* f. sp. *cepae*. The bags were incubated at 28°C for 10 days.

Plastic pots were filled with sandy loam soil at 350 g per pot. The chickpea based *F. oxysporum* f. sp. *cepae* inoculum was mixed in the soil at 5 g per pot and all the pots were watered. Pots were left for one week for establishment of inoculum. After 7 days, dried powdered leaf material of *C. didymus* was mixed in soil at 1%, 2% and 3% w/w. Pots of positive control were prepared by inoculating them with the fungus *F. oxysporum* f. sp. *cepae* only whereas pots of negative control were without inoculum and powdered leaves. The treatments were replicated three times with 10 pots in each replicate. Pots were watered and kept for 10 days.

Set bulbs of uniform diameter (3–4 cm) were surface sterilized and one set bulb was sown in each pot. The pots were watered whenever required. The pots were arranged in a completely randomized design and put under natural environmental conditions. Plants were harvested after 45 days of sowing. Plants were carefully uprooted and soil was removed from roots. Plants were washed under tap water and excess moisture was dried under fan. Data regarding disease incidence, plant mortality and shoot length were recorded. Root and shoot were separated, dried at 70°C and weighed.

Plant Physiological Tests

Chlorophyll estimation was done by method of Arnon (1949), using U2001 spectrophotometer (Hitachi-Tokyo, Japan). Sugar content was determined following Nelson (1944), using glucose as standard. Relative water content (RWC) was estimated according to Wheatherley (1950) using the following equation:

$$RWC = [(\text{fresh mass} - \text{dry mass})/(\text{saturated mass} - \text{dry mass})] \times 100$$

Membrane stability index was assessed by recording electrical conductivity of the leaves before and after heating at 100ºC (Sairam, 1994). Estimation of total phenols was performed by the method of Bray and Tharpe (1954). Total soluble protein content was estimated by method of Lowry *et al.* (1951), using Bovine Serum albumin as standard. The concentration of the unknown sample was calculated with reference to the standard curve on a fresh weight basis (Bates *et al.*, 1973). Activities of antioxidant enzymes were evaluated following Maehly and Chance (1967) using standard curves.

Statistical Analysis

Standard errors of means in laboratory and pot trials were calculated. All the data were analyzed by analysis of variance (ANOVA) followed by Tukey's HSD test using computer software Statistics 8.1.

Results

Screening Bioassays

Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem and root extracts of *C. didymus* is given in Table 1. It revealed that the antifungal effect of different parts of the plant (P) as well as extract concentrations (C) was significant for fungal biomass. Likewise, the interactive effect of P × C was also significant for the studied parameter.

The highest fungal biomass (375 mg) was recorded in control treatment. In general, all the concentrations of the three types of extracts significantly (P≤0.05) reduced the fungal biomass over control. There were 60–86%, 69–85% and 73–87% reduction in fungal biomass due to various concentrations of leaf, stem and root extracts of *C. didymus*, respectively (Fig. 1).

Effect of Soil Amendment on Basal Rot Disease

There was no disease in negative control. The highest disease incidence of 47% was recorded in positive control where only *F. oxysporum* f. sp. *cepae* was inoculated in the soil. Soil amendment with 1% dry leaf biomass of *C. didymus* reduced the disease incidence to 40% that was insignificantly different from positive control. Higher doses of dry leaf biomass exhibited pronounced and significant effect on disease incidence. Disease incidence in 2% and 3% dry leaf biomass treatments was 13% and 3%, respectively, that was significantly lower than the positive control (Fig. 2A).

The highest plant mortality of 17% was recorded in positive control and in 1% dry leaf biomass amendment treatment. Further increase in dry leaf biomass dose to 2% and 3% reduced the plant mortality to 0% (Fig. 2B).

Table 1: Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf, stem and root extracts of *Coronopus didymus* on biomass of *Fusarium oxysporum* f. sp. *cepae*

Sources of variation	df	SS	MS	F values
Treatments	23	921000	40043	81**
Plant parts (P)	2	16169	8084	16**
Concentration (C)	7	889033	127005	257**
P × C	14	15797	1128	2.3*
Error	72	35650	495	
Total	95	956650		

*, **, Significant at P≤0.01 and P≤0.001, respectively

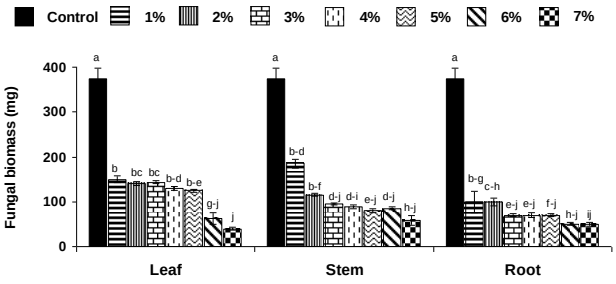

Fig. 1: Effect of different concentrations of methanol leaf, stem and root extracts of *Coronopus didymus* on biomass of *Fusarium oxysporum* f. sp. *cepae*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as Tukey's HSD Test

Effect of Soil Amendment on Plant Growth

Data regarding the effect of *F. oxysporum* f. sp. *cepae* inoculation and soil amendment with various doses of dry leaf biomass of *C. didymus* on various shoot growth parameters are illustrated in Fig. 3A and B. Inoculation of *F. oxysporum* f. sp. *cepae* significantly enhanced shoot length in positive control over negative control by 30%. Inoculation of *F. oxysporum* f. sp. *cepae* in combination with different doses of soil amendments gradually and significantly reduced shoot length over positive control. However, the difference was not significant when compared with negative control (Fig. 3A). Similar effect of *F. oxysporum* f. sp. *cepae* inoculation and soil amendments was also recorded on shoot dry weight of onion. However, difference among the various treatments was insignificant (Fig. 3B).

Data concerning the effect of *F. oxysporum* f. sp. *cepae* inoculation and soil amendment with various doses of dry leaf manure of *C. didymus* on root dry weight is presented in Fig. 3C. *F. oxysporum* f. sp. *cepae* inoculation reduced root dry weight drastically and significantly by 82% over negative control. Soil amendment with 1% dry leaf biomass of *C. didymus* alleviated the biotic stress of the fungal pathogen and significantly enhanced root dry weight by 171% over positive control. However, dry weight was

Table 2: Effect of incorporation of dry leaf biomass (DLB) of *Coronopus didymus* on various physiological parameters of plant under biotic stress of *Fusarium oxysporum* f. sp. *cepae* (FO)

Treatments	Chlorophyll content (mg g^{-1})	Sugar content (mg g^{-1})	Membrane stability index (%)	Relative water content (%)	Protein content (mg g^{-1})	Proline content (mg g^{-1})	Phenol content (mg g^{-1})	Peroxidaase activity (U min^{-1} mg^{-1} protein)	Catalaase activity (U min^{-1} mg^{-1} protein)
– Control	0.23 d	1.30 bc	85 a	97 a	1.82 bc	0.50 a	1.0 b	1.00 c	3.1 a
+ Control (FO)	0.32 c	1.44 b	36 d	45 d	1.70 c	0.48 ab	1.4 ab	1.75 b	1.4 bc
1% DLB + FO	0.55 a	1.80 a	56 b	63 b	2.23 ab	0.46 a-c	1.1 b	1.12 c	3.1 a
2% DLB + FO	0.39 b	1.67 a	43 c	51 c	2.03 bc	0.42 bc	1.3 ab	1.84 b	1.6 b
3% DLB + FO	0.28 cd	1.28 c	30 e	32 e	1.92 c	0.44 c	1.6 a	2.40 a	1.0 c

Values with different letters in a column show significant difference (P≤0.05) as determined by Tukey's HSD Test

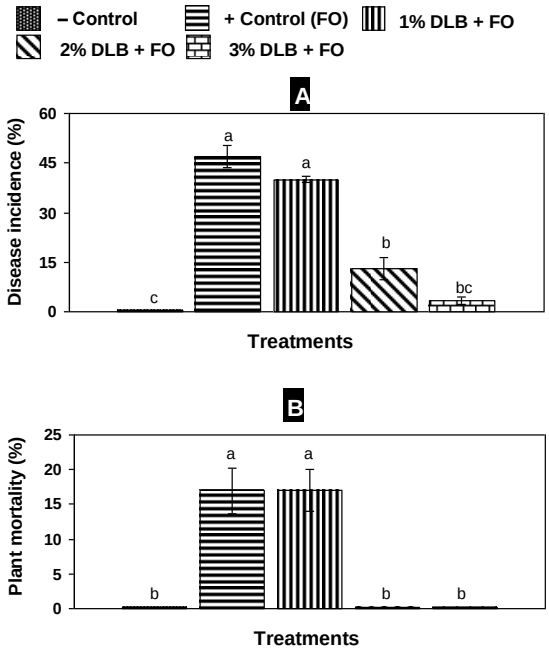

Fig. 2: Effect of application of *Fusarium oxysporum* f. sp. *cepae* (FO) and dry leaf biomass (DLB) of *Coronopus didymus* on incidence of basal rot disease of onion and plant mortality. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test

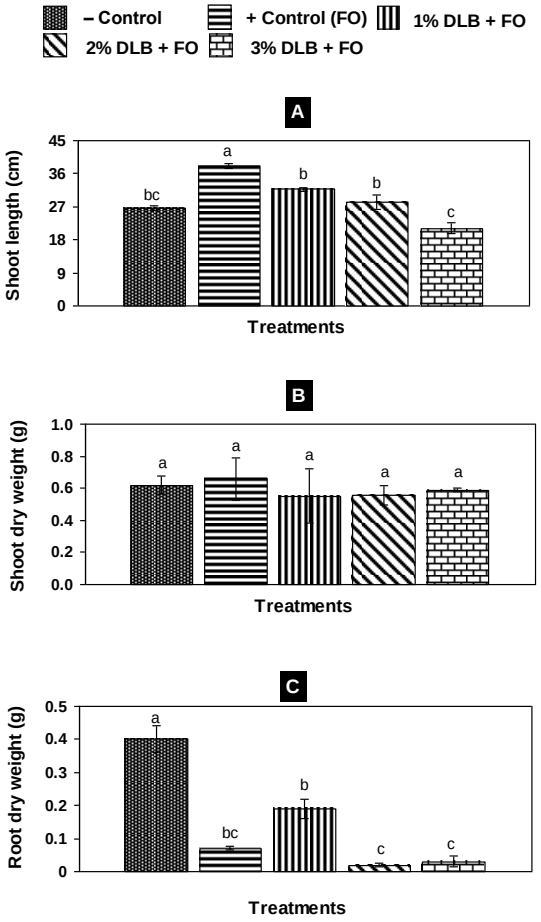

Fig. 3: Effect of application of *Fusarium oxysporum* f. sp. *cepae* (FO) and dry leaf biomass (DLB) of *Coronopus didymus* on shoot and root growth of onion. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Tukey's HSD Test

still significantly lower than negative control by 52%. Further increase in soil amendment dose reduced root dry biomass.

Effect of Soil Amendment on Plant Physiology

The lowest chlorophyll content (0.23 mg g^{-1}) was recorded in negative control. Inoculation of *F. oxysporum* f. sp. *cepae* significantly enhanced chlorophyll content over negative control by 39%. Incorporation of different doses of dry biomass of *C. didymus* to the soil significantly enhanced the studied parameter to variable extents as compared to negative control. The highest chlorophyll content (0.55 mg g^{-1}) was recorded in treatment where 1% dry biomass was incorporated. Further increase in dry biomass incorporation gradually decreased chlorophyll content in leaves of onions. Different doses of dry biomass incorporation enhanced chlorophyll content by 22–139% over negative control (Table 2).

Inoculation of the pathogen caused increased in sugar content. However, it was statistically insignificant with respect to negative control. Incorporation of 1 and 2% dry

biomass of *C. didymus* significantly enhanced the studied parameter by 38 and 28% over negative control, respectively. In contrast, incorporation of 3% dry biomass exhibited insignificant effect with respect to negative control (Table 2).

The highest membrane stability index (85%) was recorded in negative control. Inoculation of the fungal pathogen drastically reduced this parameter to 36%. Incorporation of different doses of dry biomass of *C. didymus* had variable effects on this studied parameter. One percent (1%) dose of dry biomass incorporation significantly enhanced membrane stability index over positive control by 56%. Further increase in dose of incorporated material resulted in a parallel decrease in this studied parameter (Table 2).

The highest value of relative water content (97%) was recorded in negative control. Inoculation of *F. oxysporum* f. sp. *cepae* either alone or in combination with *C. didymus* biomass incorporation significantly reduced this parameter over negative control. However, the negative effect of the fungal inoculation was less pronounced in the presence of 1 and 2% biomass incorporation of *C. didymus.* There was 54% reduction in the studied parameter in *F. oxysporum* f. sp. *cepae* alone inoculation over negative control that was reduced by 35 and 47% due to 1 and 2% *C. didymus* incorporation, respectively. The lowest value of relative water content (67% lower than negative control) was recorded due to combined application of *F. oxysporum* f. sp. *cepae* and 3% dose of *C. didymus* (Table 2).

The highest protein content (2.23 mg g^{-1}) was recorded in 1% dry biomass incorporated treatment that was significantly higher by 31% over positive control. In general, the effect of fungal inoculation as well as *C. didymus* biomass incorporation exhibited insignificant effect on protein content of onion leaves with respect to negative control treatment (Table 2).

The highest proline content (0.50 mg g^{-1}) was recorded in negative control. All the fungal inoculated treatments showed lower proline content than negative control. The adverse effect of pathogen was more severe when fungal inoculation was done in combination with dry biomass of *C. didymus* as compared to fungal inoculation alone. Proline content was decreased with increase in dry biomass incorporation. The adverse effect was statistically significant over negative control when fungal inoculation was done in combination with 2% and 3% dry biomass incorporation (Table 2).

All the fungal inoculated treatments showed higher values of phenols than negative control. The highest value of phenols (1.6 mg g^{-1}) was recorded in treatment where fungal inoculation was done in combination with 3% dry biomass of *C. didymus* that was significantly higher than phenolic content in negative control (Table 2).

Peroxidase activity showed a response to pathogen inoculation and *C. didymus* dry biomass incorporation similar to the response of phenolic content. The lowest value (1.0 U min^{-1} mg^{-1} protein) was recorded in negative control. This physiological parameter was enhanced by 12–140% over negative control in various *F. oxysporum* f. sp. *cepae* inoculated treatments. The highest value of peroxidase activity (2.40 U min^{-1} mg^{-1} protein) was recorded in treatment where 3% dose of *C. didymus* was incorporated in combination with the pathogen (Table 2).

Inoculation of *F. oxysporum* f. sp. *cepae* (positive control) significantly reduced catalase activity (CAT) by 55% over negative control. Incorporation of 1% dry biomass of *C. didymus* significantly alleviated the biotic stress of the fungal pathogen and showed catalase activity equal to negative control. Further increase in dry biomass resulted in a parallel decrease in the studied parameter (Table 2).

Discussion

In vitro, fungal biomass was significantly declined due to various concentrations of leaf, stem and root extracts of *C. didymus*. Earlier, Iqbal and Javaid (2012) reported that different concentrations of methanolic extracts of different parts of *C. didymus* reduced biomass of *Sclerotium rolfsii* by 26–67%.

In pot trial, only leaf biomass of *C. didymus* was utilized to manage basal rot of onion. It was noticed that increase in dose of leaf biomass incorporation from 1–3% gradually reduced disease incidence. Earlier, Riaz *et al.* (2012) demonstrated that soil amendment with different doses of dry biomass of *C. didymus* reduced incidence of corm rot disease of *Gladiolus grandiflorus* by 71–88%. In similar way, many studies revealed that soil incorporation of plant materials of members of family Brassicaceae managed many soil-borne phytopathogens including *Alternaria alternata, Aphanomyces euteiches* and *Verticillium dahliae* (Muehlchen *et al.,* 1990; Subbarao *et al.,* 1994; Troncoso *et al.,* 2005). Antifungal activity of brassicaceous members could be attributed to the production of secondary plant products glucosinolates (Al-Gendy *et al.,* 2010; Sun *et al.,* 2011). Enzymatic hydrolysis of these compounds by membrane-bound thioglucosidase produces many compounds including isothiocyanates, thiocyanates, epinitriles, nitriles, and glucose. Among these, isothiocyanates possess antifungal properties (Blažević *et al.,* 2010). One of the important glucosinolate in several Brassicaceous species is allyl glucosinolate that is generally broken down into allyl isothiocyanate in the soil (Mayton *et al.,* 1996). Antifungal activity of this compound is comparable to that of methyl isothiocyanate that is an active component of soil fumigants (Vaughn *et al.,* 1993).

Increase in total chlorophyll content after the pathogen infection could possibly occur owing to destruction and scattering of chloroplast ultrastructure by the action of fusaric acid, fumonisins, beauvericin, enniatin and trichothecenes secreted by *F. oxysporum* (Senthil *et al.,* 2010; Maia *et al.,* 2012). Furthermore, these fungal toxins

might alter source-sink balance of onion plant by disrupting functioning of rubisco (ribulose 1, 5-bisphosphate carboxylase) (Petit *et al.,* 2006). Incorporation of 1% and 2% doses of dry biomass of *C. didymus* significantly enhanced chlorophyll content probably due to antifungal activity in leaf powder that might have direct stimulatory effect on rubisco activity (Hibar *et al.,* 2007).

Inoculation of the pathogen with and without leaf biomass increased sugar content of plants. Lobato *et al.* (2009) also reported increase in sugar content in susceptible *Phaseolus vulgaris* infected by *Colletotrichum lindemuthianum* and revealed that amplification in activities of invertase and sucrose synthesis resulted in increase in reducing sugar that might cause insignificant effect on infected plant. It could also be attributed to induction of host resistance mechanism after pathogen infection that probably generated a sink of phtosynthate and prevented its depletion from the reminder of plant (Salt *et al.,* 1988). Increased sugar contents after incorporation of soil amendments might be related to high chlorophyll content and then increased photosynthetic rate that possibly regulate sugar content by systemic acquired resistance. Overall, activation of plant defense system could enhance the sink-strength of roots and thereby stimulate carbohydrate biosynthesis in the shoots and their transport into the roots (Abdulghader and Nabat, 2008; Ahmed and Bano, 2013).

A primary response in stressed plants is a decline in plant water potential that lead to reduction in water use efficiency (Chaum and Kirdmanee, 2009). Decline in relative water content after pathogen infection is attributed to enhancement in respiration rate and membrane injury (Agamy *et al.,* 2013). However, soil amendments increased relative water content probably due to increase in water maintenance capacity of plant (Maiceeva, 1999) that possibly improved plant resistance against *F. oxysporum* f. sp. *cepae* attacking the weak plant organs (Agamy *et al.,* 2013).

It is assumed that toxins excreted by the fungal pathogen play an important role in imbalancing water content alter membrane permeability thus induce cell membrane injury (Achor *et al.,* 1993). Incorporation of dry biomass of *C. didymus* decreased the cell membrane injury through reduction of fungal infection and/or inducing the resistance of onion plant against the infection

Significant decrease in the protein content in leaves as a result of pathogen infection might be attributed to degradation of the host proteins by the proteolytic enzymes secreted by the pathogens (Tamuli *el al.,* 2013). Increase in protein content of onion plant after incorporation of *C. didymus* biomass could be attributed to increase disease resistance in plant through systemic acquired resistance and its action on DNA-RNA synthesizing protein machinery at transcriptional and/or translocational levels (El- Khallal, 2007).

Reduction in proline levels in all pathogen inoculated treatments could be attributed to decrease proline biosynthesis, higher proline demand of infected leaves than its accumulation to overcome water stress (Aldesuquy and Baka, 1991).

In the present study, there was a gradual increase in phenolic content with the increased dose of *C. didymus* leaf biomass amendment. Substantial increase in total phenolic contents was positively proportional to the degree of plant resistance against the pathogens (Abo-Elyousr *et al.,* 2009; Senthil *et al.,* 2010). Phenolics are well known antifungal, antibacterial and antiviral compounds (Salgado *et al.,* 2008). Phenolic compounds provide disease resistance either by hypersensitive cell death or lignifications of cell walls or increased content of phenol itself toxic to pathogen (Nicholson and Hammerschimdt, 1992).

A considerable increase in peroxidase activity in all the treatments explains its positive relationship with resistance developed in plant. Peroxidase probably facilitated the oxidation of phenolic compounds to quinones that was reported to limit the fungal growth and decreased disease incidence (Emeran *et al.,* 2006; Hegazi and El-Kot, 2010). Pena and Kuc (1992) hypothesized the involvement of peroxidase in the oxidation of hydroxycinnamyl alcohols to yield lignin and crosslinking isodityrosine bridges in cell wall. They also stated peroxidase produces free radicals and hydrogen peroxide which are toxic to many microorganisms.

Chemicals produced *by F. oxysporum* may induce the suppression of catalase activity, which beside other mechanisms alleviated the level of ROS and other peroxides (Balakumar *et al.,* 1996). However, accumulation of peroxides and other active oxygen species has been described as an early event in the host pathogen recognition, playing an important role in plant defense (Nafie, 2003).

Conclusion

The results of the present study conclude that *F. oxysporum* f. sp. *cepae* can be managed effectively by using extracts of different parts of *C. didymus.* In pot trial, soil amendment with 2% leaf dry biomass of *C. didymus* can effectively manage the basal rot of onion without any adverse effect on plant growth. Disease management by soil amendment with dry leaves of *C. didymus* seems possibly due to enhanced phenolic content and peroxidase activity of the host plants. Further field trials are required to confirm the results of pot trials and recommendation to the farmers.

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