Indian forests have very low growing stock (65 Mm³ ha⁻¹ yr⁻¹) and a mean annual increment of about 0.7 m³ ha⁻¹ yr⁻¹. The condition of forest based industries is not healthy in India. Therefore, promotion of large-scale farm forestry/agroforestry plantations is essential. In order to sustain the increasing demand for wood products, foresters are turning to fast growing species and plantations (Pitre et al., 2006). While, the demand for forest products and services in India is increasing, the area and quality of forests is declining due to various factors including insect pests and diseases (Mohan and Manokaran, 2013).

Poplar (*P. deltoides*) based agroforestry system is one of the viable, alternate land use systems to prevent land degradation and obtain biological production on sustainable basis in the irrigated agro-ecosystem of India (Pandey, 2007; Zomer et al., 2007). The multipurpose characteristics, i.e., fast growth, successful intercropping and market acceptability has made poplar cultivation popular among the farmers as a viable alternative to wheat-paddy rotation in north-western states of India (Chauhan et al., 2012). The species has been grown by farmers as boundary or block plantation which improves various properties of soil through addition of organic matter (Coleman et al., 2004; Singh & Sharma, 2007) and provides alternate sources of income and employment to the rural poor (Ballooni, 2003; Puri and Nair, 2007).

An area of 312,000 ha is planted under *P. deltoides* in the country, out of which 60 per cent is block plantations and 40 per cent bund plantations (ICFRE, 2012). The tree is harvested at a short rotation of 7–10 years which provides a yield of 150-200 m³ ha⁻¹ in block plantations and 12-20 m³ ha⁻¹ in boundary plantations (Kishwan and Kumar, 2003). Presently, six to eight years old poplar trees, with girth measuring 1 m at breast height (1.37m), fetches about Rs. 200,000 ha⁻¹. In this way, poplar plantation is an economically excellent alternative in increasing tree cover (http://www.wca2014.org, 2014).

Vegetative propagation is one of the distinctive characteristics of the genus *Populus* (Dickmann, 2001). Many poplar species can be hybridized easily and propagated from cuttings for genetically improved growth and disease resistance material (Bannoun et al., 1984). The means of asexual reproduction and the extent of clonality, however, differ dramatically among species (Braatne et al., 1996; Schweitzer et al., 2002; Rood et al., 2003, 2007).
Fungal pathogens are among the major bioagents which invariably cause heavy damage to poplar in nurseries and plantations. Due to worldwide shortage of timber, numbers of poplar breeding programmes have been directed to produce clones characterized by superior growth and resistance to pests (Lone et al., 2013). In India, *P. deltoides* is mainly grown as clones in association with agriculture crops. Ninety per cent of the total planted poplar in India comprises clones like G-48, Uda, WSL-22, WSL-32, WSL-39, Wmco81 and S7C15 in the states of Punjab, Haryana, Uttarakhand and U.P. Woodlots and forest plantations, especially monocultures of genetically similar trees (clone), are highly vulnerable to insect pests and diseases (Wingfield et al., 2001; Cock, 2003). Disease problems have, therefore, posed the question regarding the overuse of single clones and preference of large monoclonal plantations (Stelzer & Goldfarb, 1997).

Presence of insect pests and diseases can cause reduction of increment and vitality in fast growing hybrid poplar plantations (Cellerino & Gennaro, 1999). Exotic plantation forestry has benefited from high productivity which is linked primarily to the absence of pests in native ranges (Bright, 1998). Although, many pathogens have gradually appeared in these plantations; still, losses have been small relative to what they might have been. Poplar was introduced in India in 1950 from USA. The common belief that plantations of non-native trees may also be more susceptible to infections because they are growing ‘off site,’ i.e., under suboptimal conditions (Brasier, 1995) seems valid as new species of fungi are regularly noticed on poplar in India.

*P. deltoides* has been first time reported as a host for several fungi (Singh et al., 2012). There may be few more reasons for it besides, discussed above. First, many new clones have been tested and recommended; however, the farmers prefer to raise monoclonal plantations of their choice for different considerations including availability of plating material, higher productivity, market rates of the wood, etc. The genetic homogeneity brought out by monoculture poses a major threat as such material is vulnerable to diseases/insect pests (Chauhan et al., 2008). For example, G-48 is an approximately 50-year-old and highly disease-susceptible clone; but, most sought after and cultivated (1/3rd of the total planted area; Dhiman & Gandhi, 2012) for its high productivity. It may, therefore, also serve as reservoir for infections. Second, disease profiling and pathogen succession and diversity are poorly attempted and studied areas. More efforts with time may bring out new facts about the
pathogens of this ecologically and commercially important tree. Further, only resistant clones can be a viable, eco-friendly and economic alternative to such problems.

During field surveys of poplar nurseries during 2008 to 2012, a leaf spot disease was observed on almost all commercial clones of *P. deltoides* (G-48, Udaipur, WSL-22, WSL-39, S7C8, etc.) that was akin to Curvularia infection. When leaf spot samples were isolated on PDA, it was identified as *Curvularia* sp. *Curvularia* is, otherwise, known to be a weak pathogen (Butler, 1953; Hodges and Campbell, 1995; Kilambi, 2005; Smiley et al., 2005). The possible reason for the shift of pathogen from weak parasite to epidemic proportions may be attributed to the change in temperature and other climatic factors that activate some sleeper pathogen species (like *Curvularia*) while, other may cease to be economically important (Chakraborty et al., 2008).

High disease incidence on an important agroforestry tree led to consideration of working out the morphology and physiology of the casual pathogen for its effective management. It is quite possible that variability in morphology, physiology, pathogenicity and so forth are mechanisms for the fungus to have better adaptation in response to diversified environment. It also leads to development of resistance genotypes of different crops against diseases (Mayek-Perez et al., 2001; Su et al., 2001).

No detailed investigation of the Curvularia leaf spot has been made previously in India. Roving survey was conducted in poplar nurseries situated at Haryana, Uttarakhand and Uttar Pradesh for the collection of *Curvularia* sp. isolates from the different clones. The disease was usually seen during monsoon season from July to late September. But the symptoms reached their peak from early-August to late September. The presence of the disease during this period may be attributed to the favourable temperature and humidity for the survival of pathogen. Gupta et al. (2001) and Panwar (2010) also recorded high incidence of Curvularia leaf spot on *P. deltoides* from early August till late September. It is an established fact that for each fungus, there is a minimum, optimum and maximum temperature for growth and sporulation. Studies revealed that temperature ranging from 25°C to 35°C was better for the growth of *Curvularia* spp. The weather data (2008-2012) of all surveys sites indicated that average temperature from July to October ranged from 30.6°C to 32.4°C (max.) to 22.7°C to 24.8°C (min.). This temperature range matches with the optimum temperature requirements for the growth of *Curvularia* sp. and may be possible reason for large scale presence of the disease.
Sixty isolates of *Curvularia* sp. were collected from different clones. Isolate collection practically matched clones distribution in India (Fig.2.1.4.), i.e., maximum numbers of isolates were collected from clone G-48 (19) and minimum from WSL-39 (4; Fig.4.1.2.). Udaï and WSL-22 contributed 14 isolates each of *Curvularia* sp. This may be due to the fact that G-48 was most preferred clone and, accordingly, occupied largest per cent share in the nurseries. It covered 36.2 out of 90 percent areas under *P. deltoides* in north-western states of India (Dhiman and Gandhi, 2012). Moreover, presently, this clone is most susceptible to diseases leading to high isolate recovery.

In plant pathology, suggestions of control measures depend on proper identification of disease and of the causal agent. Often, plant pathologists have to rely on symptoms for the identification of a disease problem (Riley *et al.*, 2002). However, the use of symptoms alone is often an inadequate method for disease identification. Thus, for proper management of the disease, it is necessary to have defined symptoms of it. So in the present study, symptomatology of the leaf spot disease was defined in details. Curvularia leaf spot symptoms were more severe on middle and lower (old) leaves of the seedlings (7 to 8 month old) which were near to soil. It is reported that high fungal counts on old leaves are associated with low content of tannins and sugars, high level of amino acids, low leakiness of tannins and sugars, and high leakiness of amino acids (Sivakumar and Kathiresan, 1990; Ravikumar and Kathiresan, 1993); hence, easy to get infected with plant pathogenic fungi (Mamza *et al.*, 2008). Moreover, the high moisture content surrounding the lower leaves improves the growth and spore germination of plant pathogenic fungi (Cohen *et al.*, 1971), therefore, increase in the severity of the disease. The symptoms of Curvularia leaf spot on *P. deltoides* showed small, yellow brown, necrotic spots of varying shape on leaf surface and later, they turned light to dark brown surrounded by a yellow halo (Fig.4.2.1.A & B). Presence of spots surrounded by a yellow halo is an important symptom of the disease (Fig.4.2.1.C & D). It is believed that, a fungal specific toxin may be responsible for the halo (http://www.plantprotection.hu, 2014). Similar symptoms with frequent haloing were also recorded by Panwar (2010) on the leaf blade *P. deltoides*.

The pathogenicity of the fungus was proved by artificial inoculation of *P. deltoides* clones with the conidia of the pathogen. In the present study, spray with conidial suspension was used as inoculation method which resulted in symptom
Leaf spots were initially observed on lower leaves as found in the field. Symptoms developed on leaves during pathogenicity were comparable with the field (Table 4.3.1.). Time of symptoms appearance varied among clones. Symptoms first appeared on G-48 (18 d) and then on WSL-22 and WSL-39 (18 to 20 d) while, it took 20 days on Udai (Fig. 4.3.1.). The high susceptibility of the G-48 clone may have led to earliest symptom appearance on it.

In India, *C. lunata* was reported on *P. deltoides* (Gupta *et al.*, 2001). In the present investigation, on the basis of morphological characters, *C. eragrostidis* was identified which is new on *P. deltoides* (Bagwari *et al.*, 2014). Emerging new diseases represent a growing worldwide problem accompanying global environmental changes. The diseases can also result from a sudden increase in virulence and/or expansion of the geographic range of a previously unnoticed pathogen (Fisher *et al.*, 2009). Being able to identify species more likely to become invasive or turn into emerging diseases would greatly facilitate prevention tasks (Enserink, 1999). There is tremendous interest in identifying the factors controlling the appearance and spread of these diseases (Giraud *et al.*, 2010). Accurate and timely reports of new host-fungus records are essential for diagnostics, identification, management and prevention of plant diseases (Dugan *et al.*, 2009).

Good indicators of virulence include factors like growth rate, sporulation and germination (Chandler *et al.*, 1993; Altre *et al.*, 1999). Growth and sporulation are known as universal virulence factors in disease development. So, fifteen isolates were selected on the basis of growth rate, sporulation and crude toxin weight (considered as part of pathogenesis) from the total population for further studies. The two methods, namely, manual and numeral were tried and it was observed that barring three isolates, no. C-38, C-43 and C-54 of numeral method, rest of the isolates were common in both the methods (Table 4.5.2.1.). Further, a phenogram was also constructed and all selected isolates of *Curvularia* sp. fell in to one major cluster divided into two sub-clusters, i.e., nine isolates (no. C-10, C-21, C-27, C-28, C-33, C-35, C-36, C-37 & C-40) were grouped in cluster IA while, remaining six isolates (no. C-2, C-5, C-29, C-34, C-39 & C-56) fall in cluster IB (Fig. 4.5.3.1.). It signifies the similarity among these selected isolates.

Fungi secure food and energy from the substrate upon which they live in nature. It is well known that the fungal pathogens attack the host plant for their nutritional requirements. In order to culture fungus in laboratory, it is necessary to
furnish essential elements and compounds in the medium for their growth and other life processes (Ramjegathesh and Ebenezar, 2012). The nutritional requirements of fungi differ and there is no single medium which may be universally suited for the growth of all fungi. Therefore, for experimental work in laboratory first, the pathogen is grown on different media and, then, most suitable medium selected (Chaurasia et al., 2013). In the present study, four different media, viz., MEA, PDA, SDA and WA were tested with regards to morphological characters, rate of growth, sporulation and spore size. Varied colony types (Cottony, effuse, flat, floccose, velvety, woolly & velutinae) were observed for the isolates of Curvularia sp. on different growth media tested (Table 4.6.1.1.; Fig.4.6.1.1. to 4.6.1.6.). Woolly colonies were predominant on all the four media as supported by five isolates on MEA, three isolates on PDA and SDA, respectively and nine isolates on WA. Cottony colony was second to woolly. The findings also matched with the work of Ellis (1971) and Kaur et al. (1973) who reported various colony textures (effuse, cottony, velvety, woolly & hairy) of the pathogen.

Grey colour colonies were recorded by majority of the isolates of Curvularia sp. on four different media. Further, on MEA three isolates (C-2, C-5 & C-10), on PDA two isolates (C-2 & C-33), on SDA four isolates (C-2, C-5, C-10 & C-27) and on WA three isolates (C-29, C-35 and C-36) had other than grey colour colonies (Table 4.6.1.2.; Fig.4.6.1.1. to 4.4.6.6.). According to Ellis (1971), young colonies of Curvularia spp. were greyish-brown becoming dark brown with age; reverse of colonies were greyish brown. Colonies of C. eragrostidis were also found blackish grey by Salleh et al. (1996).

The pigmentation can be observed as change in colour of the growth medium. Like colonies colour, different shades of grey were dominant in pigmentation (Table 4.6.1.3.; Fig.4.6.1.1. to 4.6.1.6). On MEA and PDA, grey colour pigments were released by majority of the isolates (13 & 14 isolates, respectively). Different trends were observed on SDA like buff pigments were released by six isolates. In contrary, six isolates of Curvularia sp. (C-28, C-33, C-35, C-37, C-39 & C-56) did not release any type of pigment on SDA. Olive pigmentation was predominant on WA like, greyish olive. Presence of grey (dark) colour in colonies and pigmentation confirm that the pathogen belongs to dematiaceous group of fungi which have characteristic dark colour (labmed.ucsf.edu/education/residency/fungal site/dematpage.html, 2014).
The relative growth of *Curvularia* sp. isolates on different media showed differential pattern after 8d of fungal inoculation when isolate no. C-28, C-34, C-35 and C-39 filled MEA Petri plates completely (7.0cm; Table 4.6.2.1.). MEA supported significantly more and maximum growth (6.2 cm) among all media tested followed by WA (5.8 cm). Minimum growth was recorded on SDA (4.0 cm). In contrary, Sumangala and Patil (2010) found PDA the best and MEA poor medium for the growth of *C. lunata*. Determination of optimum growth period is essential to study the physiology of fungi (Thangamani, *et al.*, 2011). Average time taken by all the isolates to complete their full growth differed among media (Table 4.6.2.3.). Fungal isolates took average time of 5.9d to complete growth on MEA which was quite less and close to 6.1d of WA. On the other hand, isolates, on an average, finished the growth in 7.7d on PDA. None of the isolates could fill the Petri plate on SDA even after 10d of observation.

Growth increments of *Curvularia* sp. isolates were plotted over time, it was observed that all the isolates instantaneously picked up growth on all four media after 24h (Fig.4.6.2.1. to Fig.4.6.2.4.). Afterwards, the growth declined gradually with the increase in number of days of incubation. It may be possibly due to autolysis of the fungus and exhaustion of nutrients in the medium as opined by Lilly and Barnett (1951), who also pointed out that the growth of the fungus, as in other organisms, follow a definite pattern which depend on species, environmental and nutritional conditions. In the present case, all isolates exhibited sharp declined of growth on MEA and SDA after 5th day and 3rd day, respectively. While, on PDA and WA the autolysis was recorded after 7th and 6th day of incubation, respectively.

The reproductive phase of fungal life cycle, sporulation was best supported by PDA (Table4.6.3.1.). Moderate sporulation was recorded on MEA and WA. These trends partially matched with the finding of Olufolaji (1996) who also studied effect of eight different media (PDA, WA, CMA, MEA, NA, YEA, DA & PA) on the growth and sporulation of *C. pallescens*. MEA and PDA were found best for sporulation of the fungus while, WA was found poor medium for the sporulation. The results were also in the accordance with the work of Sumangala and Patil (2010) who found PDA as best medium for the sporulation of *C. lunata*.

Conidia size differed on growth media used. Barring on PDA, isolate no. C-34 had largest conidia on three media, i.e., MEA, SDA and WA (Table4.6.4.1.). While, isolate no. C-39 registered maximum size of conidia on PDA. The size of conidia on
all four media was comparable with conidia size reported by Ellis (1971). Morphological characteristics of conidia and conidiophores and, sometimes, host plant association provide the major taxonomic criteria for delimitation of fungal species (David, 1991). However, the classification of small spored species, including host-specific toxin producing fungi, has been particularly confused, because of the simple and convergent morphology of conidia and facultative parasitism, resulting in an ambiguous host range (Simmons, 1999). Outliers and extreme values were observed for length and breadth of *Curvularia* sp. isolates when box plots were drawn (Fig. 4.6.4.1. to 4.6.4.4.). Isolates exhibited maximum number of outliers (2.2%) on the upper side of plot for the conidia length on PDA. While, it was 2.7 per cent for upper limit of the plot for breadth on SDA. On the contrary, there was no outlier for breadth of isolates on MEA. *Curvularia* sp. isolates exhibited maximum number of extreme values (4.2 %) on upper limit of length on WA (Fig. 4.6.4.4.). Like outliers, no extreme value for the conidial breadth was observed on MEA.

Cochrane (1958) reported that any consideration of ecology or spread of the economically important fungi must take spore germination into account. The term conidia germination in fungi consists of all processes that occur during the development of a dormant structure in to a vegetative cell. The definition of fungal spore germination is not consistent but generally conidia said to have germinated when the length of germ tube is about 2/3rd of the length of the conidia (Dhingra and Sinclair, 1985). In the present work, isolate no. C-34 took maximum time (7 hr) to initiate the germination of conidia. While, minimum time of 4 hr was recorded by six isolates (Table 4.6.5.1.) Majority of the isolates (9) showed bipolar type of germination.

In the present study, MEA was found best medium for the growth of *Curvularia* sp. isolates whereas, PDA was found best for the sporulation of the fungus. Sumangala and Patil (2010) also observed that an increase in radial growth did not result in simultaneous increase in conidial yield. The mycelial growth and conidial yield on artificial media depends upon the fungal isolates and the constituents used in the culture media (Mustafa and Kaur, 2009).

Nutrients are the substances used in biosynthesis and energy release, therefore, serve as cardinal impetus towards the viability, survival and sustenance of any organism (Safavi *et al.*, 2007). A thorough knowledge of the nutrition of the pathogen has a basic significance in understanding the parasitism on the host which involve a
nutritional relationship between the two. Further, the resistance and susceptibility of the host is considerably determined by what the host has to offer by way of nutrients; because the successful pathogenesis of a parasite must obtain at site of its localization on the host the kinds and amount of various nutrients for its proliferation (Gemawat and Prasad, 1971). The kinds of nutrients the pathogen utilise in vitro, may indicate what it takes from the host plant. Different nutrients were used in the present study to check the suitable nutrients for the growth of the fungus. PDB with and without Poplar Leaf Extract (PLE) was used for the pathogen growth. PDB with PLE showed significantly higher dry mycelium weight than PDB alone (Table 4.7.1.1.). It is believed that natural media (vegetable and plant extract host decoction) stimulates the growth of fungus (Patil and Rao, 2003). It has also been observed that the sub culturing of a fungus over a period of time leads to loss of many characteristics including pigmentation. Therefore, periodic mixing of host extract in the medium during sub culturing is one of the strategies to revive the fungal characteristics. Probably, the complex of constituents of the host extract offers some nutrients that, otherwise, are lacking in the synthetic medium. That is why PDB with PLE performed significantly better than PDB alone.

Carbon occupies a unique position among the essential elements required by living organisms. As a component of both structural and functional constituent, carbon comprises about 50 per cent of the total mycelial dry weight in fungus (Bilgrami and Verma, 1978). Every fungus has specific nutrient requirements for carbon and nitrogen in their growth, infection and reproduction (Sarbhoy, 1965; Sangeetha and Rawal, 2008). Among various carbon compounds tested in the present study, sucrose (control) supported maximum growth of the fungus. Second to it, glucose had maximum and significantly high dry mycelium weight. It was very interesting to note that none of the Curvularia sp. isolates supported any growth on tartaric acid. Further, significantly low dry mycelium weight was recorded on sorbose (Table 4.7.2.1.).

The variation of growth due to different carbon compounds may be attributed to difference in permeability of cell wall or to presence or absence of specific enzyme necessary for the respiratory steps followed by that compound during its assimilations. It is evident from the present investigation that except tartaric acid, the fungus was able to utilize xylose, glucose, sorbose, maltose, starch and mannitol when they were replaced individually as carbon sources in the basal medium. These trends
are slightly varied from the previous results obtained by Bais et al. (1969) where in out of ten carbon sources tested, glucose supported maximum growth of *C. pallescens*. However, all isolates of *Curvularia* sp. exhibited growth second to sucrose (control) in glucose. It is well established fact that disaccharides (sucrose) is of common occurrence in plants. Bilgrami and Verma (1978) also opined that sucrose, being the major sugar component of photosynthetic plants, is generally utilized as prefered carbon source by most of the plant pathogenic fungi. A large number of workers have shown that most of the fungi are able to hydrolyse sucrose into glucose and fructose and, thus, it is assimilated through a hydrolytic pathway (Nagadesi and Arya, 2013). Similarly, the findings are close to report of Madan and Thind (1998) who reported that, among disaccharides in general, sucrose and maltose have shown to be excellent or good source of the carbon for the growth of the fungi. Organic acid like tartaric acid, in general, was reported as poor sources of carbon for the growth of fungi (Madan and Thind, 1998).

Nitrogen is an essential element for fungi. It is not only required for functional and structural purposes but also constitutes a fraction in vitamin and other essential metabolites (Bhargava, 1968). Nitrogen is a very important element in the protein synthesis also. Further, the adequate source of nitrogen also plays an important role for good mycelial growth of fungi (Zamir and Hussain, 1970). But all the sources of nitrogen are not equally good for the growth of all the fungi (Lilly and Barnett, 1951). In the present study, among the various nitrogen sources tested, sodium nitrate (control) was found best for the growth of *Curvularia* sp. isolates, and minimum dry mycelium weight was recorded in sodium nitrite (Table 4.7.3.1.). These results are partially similar to the results of Dandge, (2012) who found potassium nitrate supported excellent growth of *Curvularia* spp. However, the present findings do not tally with the work carried out by Bais et al. (1969) who reported peptone to be best for the growth and sporulation of *C. pallescens* out of 19 nitrogen compounds tested. Nitrite is found to be toxic for most of the fungi and the poor growth of many fungi in nitrite was attributed to the toxic effect exerted by the pyruvic acid accumulated in the mycelium (Nord and Mull, 1945).

Magnesium sulphate (control) and potassium sulphate were found best for the growth of *Curvularia* sp. isolates. While, zinc sulphate was found to be poorest sulphur source for the growth of *Curvularia* sp. isolates (Table 4.7.4.1.). The trends exactly matched with the results of Hasija, (1967) and Bais et al. (1972) who found
magnesium sulphate supported best growth and sporulation of *C. pallescens*. Several workers (Armstrong, 1921; Mosher *et al*., 1936; Tandon, 1950; Hasija, 1967) also had reported sulphate to be good sources for various fungi among different sulphur compounds. Zinc sulphate was found to be poorest sulphur source for the growth of *Curvularia* sp. isolates. Carbon, nitrogen and sulphur constituents of CDB was found best for the growth of *Curvularia* sp. isolates as maximum dry mycelium weight was recorded in sucrose, sodium nitrate and magnesium sulphate, respectively. It re-establishes the suitability of CDB for the growth of *Curvularia* sp. of poplar.

The environmental factors such as incubation period, temperature, pH, etc. not only affect the development and spread of fungal disease but also greatly influence the growth and sporulation of pathogens (Mishra and Hoque, 1962; Mathur and Sorbhooy, 1976; Sharma and Kaushal, 1979; Singh and Sandhu, 1982; Khan and Quazi, 2010). Variations in pH and temperature lead to differences in growth and reproduction and, therefore, for each fungus there is a minimum, optimum and maximum of these two factors (Tandon and Mitra, 1963; Lilly and Barnett, 1951; Cochrane, 1958; Chauhan and Suryanarayana, 1970; Bilgrami and Verma, 1978). Hydrogen ion concentration of the medium has a profound effect upon the rate and amount of growth and many other life processes of the fungus (Lilly and Barnett, 1951). In the present study, maximum dry mycelium weight of *Curvularia* sp. isolates were recorded at 6 pH (Table 4.8.1.1; Fig. 4.8.1.1). Further, higher fungal growth was recorded at higher range of pH till pH 12 contrary to no growth at lower pH of 2. These trends are supportive of reports in literature of best growth of fungi like *Curvularia* sp. in the present case at acidic pH (of 6), (Madan and Thind, 1998) however, the results also indicate its tolerance to pH as high as 12. At reduced pH, cell membrane becomes saturated with the hydrogen ions that limit the passage of cations. The reverse could be obtained when medium are alkaline and accumulated hydroxyl ions preventing the passage of essential anions (Thangamani *et al*., 2011). Further, for many fungi, a pH range of 5.5 to 6.5 has been found to be quite suitable for their maximum growth and sporulation. Similarly, the pH of most plants ranges from 5.0 to 6.5 which obviously favoured the establishment of parasites in them. Nevertheless, the minimum, optimum and maximum pH values for their growth and sporulation of individual fungi may vary considerably (Madan and Thind, 1998).

Temperature is the most important environmental factor for regulating vegetative and reproductive activity of the fungi. Maximum dry mycelium weight was
recorded at 25°C while, minimum and significantly low dry mycelium weight was recorded at the lowest temperature of 10°C (Table 4.8.2.1.; Fig. 4.8.2.1.). Similar trends were observed by Yang (1973) and Sumangala and Patil (2010) who found 25°C and pH 6 supported maximum growth and sporulation of Curvularia spp. The optimum growth temperatures for majority of fungi studied was found to fall between 25°C to 30°C (Sharma and Razak, 2003).

Chemical control is a fast and effective method of fungus management. Fungicide application can minimize disease and, thus, increase the genetic potential and ultimately yield. Fungicides may act on or interrupt the metabolic system of the pathogen (Bilgrami and Dubey, 1976). The effectiveness of a fungicide depends on its innate toxicity and permeation. However, there are reports of resistance development against fungicides. Therefore, judicial use of fungicides at proper time is necessary. Two fungicides, namely, Propiconazole (systemic) and Dithane M-45 (non-systemic) were tested in vitro against Curvularia sp. isolates. Propiconazole was more effective in suppressing the growth of the fungus than Dithane M-45 as cent per cent inhibition was achieved for all the isolates at lower concentration of 12ppm against higher concentration of 150 ppm of Dithane M-45 that too excluding one isolate no. C-2 (Table 4.9.1.1; Fig. 4.9.1.1. to 4.9.1.3. & Table 4.9.2.1; Fig. 4.9.2.1 to 4.9.2.3.). Non-systemic fungicides prevent infection largely by inhibition of spore germination and germ tube elongation. The increased concentrations probably give a better effect (Iqbal et al., 2010). In vitro evaluations of four fungicides (organomercurial, benlate, mancozeb & propiconazole) by Olufolaji (1996) revealed that Propiconazole totally inhibited mycelial growth and spore formation in C. cymbopogonis at 5 g/l (5000 ppm). The results are also accordance with Gupta et al. (2014) who found Dithane M-45 (non-systemic) more effective than Bavistin and Bayleton (systemic) to control the C. lunata at 50, 100 & 200 µg/ml. Similar observations were also recorded by Falloon (1975) who tested six fungicides against C. trifolii and found Dithane M-45 most effective against the pathogen.

Biological control through the use of antagonistic microorganisms is a potential, non chemical means of managing plant diseases by reducing inoculum level of pathogens. Such a management would help in preventing the pollution and also health hazards. The antagonism of Trichoderma spp. against many fungi is mainly due to production of acetaldehyde, a carbonyl compound (Robinson and Park, 1966; Dennies and Webster, 1971). In the present investigation, T. harzianum exhibited
significantly more growth inhibition as compared to *T. viride* though, quite low (16.7 % & 15.1 % respectively, Table 4.10.1.1.; Fig. 4.10.1.1. to 4.10.1.6.). However, isolates exhibited differential sensitivity to the antagonists; for example, seven isolates had maximum growth suppression by *T. harzianum*. While, five isolates were inhibited maximally by *T. viride*. *T. harzianum* could be assigned as slightly better biocontrol agent than *T. viride*. But it was very interesting to note that both the antagonist did not showed 50 per cent growth inhibition. Therefore, these antagonists may not be recommended for the biological management of *Curvularia* sp. Contrary to the present observation, Tapwal *et al.* (2011) reported 44.3 per cent growth inhibition of *C. lunata* by *T. viride*.

During collection of *Curvularia* sp. isolates in poplar nurseries, a good number of *Alternaria* sp. isolates were also recovered. It may be due to sharing the same foliage by these pathogens over a period of time. The study was aimed to know the initiation as well as progress of the disease (s) under natural conditions when two or more pathogens are competing for the same source of food and shelter. Fifteen isolates of *Curvularia* sp. were tested against three isolates of *A. alternata*. These three isolates were selected on the basis of their growth rate, viz., A-16 (fast), A-69 (moderate) and A-49 (slow). Most of the *Curvularia* sp. isolates followed overlapping patterns in relation to growth inhibition. Fast growing isolates of *A. alternata* (A-16) had significantly more growth inhibition (14.9 %) than other two isolates of *A. alternata* which exhibited mutually at par growth suppression (13.6% each; Table 4.10.2.1; Fig.4.10.2.1 to 4.10.2.3.). Antagonist isolates (co-habitant) had outsmarted the pathogen as it suppressed all the isolates of *Curvularia* sp. to different degree but quite moderately. However, no pattern in relation to growth suppression of *Curvularia* sp. isolates vis-a-vis growth habit of *A. alternata* isolates could be drawn. Only six isolates (C-10, C-21, C-27, C-28, C-36 & C-37) exhibited loosely defined suppression in relation to growth habit of antagonist underlining the fact that interactions were highly individualistic, for example, slow growing isolate of *A alternata* (A-49) exhibited highest suppression of 25.1 per cent of isolate no. C-2; while, minimum growth suppression was recorded by moderate growing isolate of *A. alternata* (A-69) for the same isolate of *Curvularia* sp. (3.0%). Therefore, other approaches besides growth suppression need to be employed to draw meaningful conclusion from these kinds of interactions.
Selection of most virulent isolates of *Curvularia* sp. for the future screening of resistance of germplasm against the disease involved two steps. Initially, box plot analysis was employed to find out the potential interactions among the 15 isolates. The criterion for the selection was based on PIMG values of the interactions between any two isolates that could show marked superiority over their counterparts (a point in the graph where minimum value of one interaction was more than maximum value of another) when these interactions were plotted against per cent inhibition (Fig.4.10.3.2.). It was considered a benchmark for selecting out those interactions (43 in number) that fall above this point for further analysis based on DMRT (Table.4.10.3.2.). This approach was helpful in sorting out the potential interactions for further analysis out of a very large number of interactions. Still, 17 groups of interactions were formed by DMRT analysis signifying that the interactions were highly overlapping meaning, thereby, that isolates were very much comparable mutually in terms of their growth suppression potentials.

Barring five isolates (C-33, C-34, C-39, C-40 & C-56) that exhibited negative interactions, rest of the *Curvularia* sp. isolates exhibited positive growth inhibition underlying superiority of designated isolate as antagonist over the pathogen isolate (Table.4.10.3.1.). Maximum growth suppression was recorded for isolate no. C-2 by isolate no. C-27. However, the growth inhibition was less than 50 per cent. The better performance of isolate no. C-27 over C-2 may find reason in their growth rates as isolate no. C-2 (6.0cm) had significantly less growth than isolate no. C-27 (7.0 cm) on PDA. Isolate no. C-27 that performed best in term of growth suppression can be a potent candidate for further screening of poplar germplasm for leaf spot screening.

Several methods have been employed to manage plant diseases in crops (Hahn *et al*., 1989). But the most effective approach would be the selection and breeding for diseases resistance varieties (Nwankiti *et al*., 1987). Most of the existing techniques for selecting resistant varieties include evaluation for disease incidence and severity in the field and green houses. However, these screening procedures are very cumbersome, time consuming, labour intensive and require a large amount of land space (Amusa, 1991). The phytotoxic metabolite approach is an attempt to bypass many of these constraints using disease agents as same quantity of the metabolites will be applied to the host tissue directly.

Symptoms of many plant diseases revealed the involvement of phytotoxic metabolites which, therefore, suggest a role for toxic metabolite secreted by the
pathogen in the disease development. Pathogenic fungi and bacteria often damage their host plants by producing toxins which cause various symptoms including necrosis, chlorosis, wilting, water soaking and eventually the death of plants. One criterion of importance of a toxin in a disease syndrome caused by a pathogen is toxigenicity as often related to pathogenicity or virulence (Scheffer, 1983). Microbial toxins are metabolites produced by plant pathogens (fungi & bacteria) which play a role in host-pathogen interactions and disease expression. They are low molecular weight substances produced by some pathogens which are capable of reproducing symptoms similar to that found in natural infections on plants (Bilgrami and Dubey, 1976).

Keeping this in view, an experiment was conducted to screen resistant germplasm of *P. deltoides* against crude toxin of *Curvularia* sp. The leaf bioassay of toxin has been standardized based on concentration and volume of toxin, position and injury to leaf, etc. Necrosis was observed only at higher concentrations of toxin of 80 and 100 per cent not at lower concentrations (20 to 60 %; (Table4.11.2.1.; Fig. 4.11.2.1. to 4.11.2.2.). In case of volume of toxin, necrosis appeared at 50µL in G-48 while, the symptoms were observed at highest volume of 70 µL in WSL-22 when lower concentration of 80 per cent of toxin was used (Table4.11.3.1.; Fig. 4.11.3.1. to 4.11.3.2.). Further, the necrosis was seen on both G-48 and WSL-22 right from lowest volume of toxin (30 µL) to highest one (70 µL) when higher concentration of 100 per cent was tested. The significantly higher extent of necrosis coupled with its expression at lower volume and concentration in G-48 vis-a-vis WSL-22 reaffirms its higher susceptibility to diseases including Curvularia leaf spot. One more trend with regards to concentration deserves attention while standardising the toxin based screening method for disease resistance, i.e., the symptom expression is governed both by concentration as well as volume of the toxin tested as necrosis expressed at lower volume (30 µL onwards) in the both the clones when higher concentration of toxin (100%) was used. Therefore, 100 % toxin concentration and 70 µL of toxin volume were selected for the testing of leaf.

Initially, symptoms were observed at 24, 48 and 72 hr after toxin inoculation; however, after 48 hr there was no spread in symptom (Table4.11.2.2. & 4.11.3.2). In further experiments, the duration of observation was kept only for 24 hr after toxin inoculation. Moreover, necrosis with occasional haloing was observed in some cases. Contrary to it, Liu *et al.* (2009) observed all necrotic lesions showed halo surrounding
edges after 48 and 72 hr when toxin of *C. lunata* was inoculated on maize. Alam *et al.* (1997) obtained similar results with *C. andropogonis* a casual agent of leaf blight of *J. citronella*. Zhao *et al.* (1997) also inoculated culture filtrate of the pathogen on host leaves which caused necrotic spot similar to those by natural infection and suggested that symptoms were at least partly due to toxin produced by the pathogen.

Generally, isolates of *Curvularia* sp. took maximum time on un-pricked leaves to initiate the symptoms than pricked leaves in all clones (Table 4.12.1.; Table 4.12.2.; Table 4.12.3.; Table 4.12.4.). Isolate no. C-36 took maximum time on un-pricked leaves to develop symptom in two clones (G-48 & WSL-22) and isolate no. C-28 took minimum time to initiate symptom on Udai and WSL-39 leaves. Un-pricked leaf had minimum and significantly less necrotic area in all four clones tested (Table 4.12.1.; Table 4.12.2.; Table 4.12.3.; Table 4.12.4.). It points out that pricking (wounding) of leaf facilitate the penetration of toxin and, subsequently, symptom development. Isolates exhibited different trends on un-pricked leaves for example, eight isolates on G-48, six isolates on Udai, seven isolates on WSL-22 and four isolates on WSL-39 produced necrosis (Table 4.12.1.; Table 4.12.2.; Table 4.12.3.; Table 4.12.4.). Again, G-48 clone was found most susceptible as maximum number of isolates (8) exhibited necrotic symptom even on un-pricked leaves. Relatively, WSL-39 was found resistance as only four isolates was capable to initiate symptom on un-pricked leaf. Isolate no. C-5, C-29 and C-35 showed symptom on un-pricked leaf of all the clones tested showing their virulent nature as they could penetrate the intact leaf and develop necrosis (Table 4.12.1.; Table 4.12.2.; Table 4.12.3.; Table 4.12.4.).

Generally, symptoms initiation was earliest on upper leaf than middle and lower leaves (Table 4.12.1.; Table 4.12.2.; Table 4.12.3.; Table 4.12.4.). Further, three clones exhibited maximum necrotic on upper pricked leaf while, in case of WSL-22 middle leaf was most susceptible to disease (Table 4.12.3.2). Changes in amounts of wax between surfaces of leaves of varying ages may also affect pathogen behaviour and, consequently, affect the infection process (Martin, 1964). The leaflets probably develop some type of ontogenic resistance with aging (Popular, 1978). Thus, it is important to evaluate the influence of plant tissue phenological age on pathogen infection, in order to provide information necessary for defining control strategies. Different leaf surface topographies, which affect wax arrangement and quantity, must
also be considered. The above observation support the present trends as youngest upper leaves developed earliest symptoms.

Isolates of *Curvularia* sp. showed different pattern in terms of time of initiation of symptom on leaf positions. For example, isolate no. C-37 took minimum time to initiate the symptom on lower (Udai), upper and middle leaf (WSL-22 & WSL-39, respectively; Table4.12.1.1.; Table4.12.2.1.; Table4.12.3.1.; Table4.12.4.1.). On the other hand, isolate no. C-56 took minimum time to initiate the symptom on all three positions of G-48 clone. Among the isolates, no. C-5 had maximum necrotic area in all four clones tested (G-48:56.2%; Udai: 26.5%; WSL-22: 51.1% & WSL-39: 19.4%; Table4.12.1.2.; Table4.12.2.2.; Table4.12.3.2.; Table4.12.4.2.) supporting its primacy in virulence over the companion isolates of *Curvularia* sp. Selecting plants of similar age, leaves of similar age and position on the plants (Visker *et al.*, 2003) is important for consistent results in controlled inoculation study. The present study addresses all these concerns to achieve a reproducible testing methodology for *Curvularia* sp. toxin.

Shoot juvenile experiment was also performed to screen the *P. deltoides* clones against *Curvularia* sp. toxin. G-48 expressed necrosis in minimum time while, WSL-39 exhibited it in last (Table4.12.5.1.). Interestingly, G-48 again expressed its earliest susceptibility to the pathogen toxin. The pattern of development of necrosis was different in shoot juvenile than of leaf bioassay as it spread through veins over time (Fig.4.12.5.1. to 4.12.5.4.). Both these methods are useful for quick screening of the germplasm resistance against leaf spot of *Curvularia* sp.

Poplar, as agroforestry tree, is slowly coming up as economically viable alternative to traditional agriculture (for example, paddy-wheat rotation). However, due to limited number of clones and large scale monoclonal plantations diseases keep coming reducing poplar productivity and limiting its optional value. Emerging new diseases may also be a result of climate change and sudden change in virulence of otherwise, weak pathogens like *Curvularia* sp. For managing spurt of such diseases, knowledge of pathogen is essential both for understanding its variation as well as to draw a management strategy.

*C. eragrostidis* was discovered as a new species on *P. deltoides*. Moreover, the variability of the pathogen was captured not only at morphological and physiological level but also at selection of isolates from a large population based on an objective methodology. Even the collection of isolates was representative of clones in field.
(nurseries), geographical area, etc. making the population inclusive. Systemic fungicide was found better than non systemic and can be used to manage the disease. However, the antagonists fared poor making the option of biological disease management non viable. The interactions of *Curvularia* sp. with cohabitant, *A. alternata* and among its isolates brought out an important fact that colonisation of the host is not solely dependent on growth rate of a pathogen but also on some other factors. This observation was further supported by the deviating trends of leaf bioassay and shoot juvenile screening for the resistance of *P. deltoides* germplasm against toxin of *Curvularia* sp. As different isolates than in interaction study performed better in terms of symptoms initiation as well as development (necrosis). However, both the methods were found quick, reproducible and effective for disease screening. The clone G-48 was found most susceptible and WSL- 39 relatively resistant against Curvularia leaf spot.