

EPIDEMIOLOGY AND MANAGEMENT OF FUSARIUM HEAD BLIGHT OF WHEAT

THESIS

By

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(A-2018-40-024)**

Submitted to



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CERTIFICATE – I

This is to certify that the thesis entitled “**Epidemiology and management of Fusarium head blight of wheat**” submitted in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Plant Pathology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by Miss Gurpreet Kaur (**A-2018-40-024**) daughter of **Shri Kewal Singh and Smt. Sukhwinder Kaur** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

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CERTIFICATE- II

This is to certify that the thesis entitled “**Epidemiology and management of Fusarium head blight of wheat**” submitted by **Miss Gurpreet Kaur (A-2018-40-024)** daughter of **Shri Kewal Singh and Smt. Sukhwinder Kaur** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Plant Pathology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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LIST OF ABBREVIATIONS USED

S. No.	Abbreviation	Meaning
1	%	Per cent
2	&	And
3	/	Per
4	@	At the rate
5	µl	Micro litre
6	mt	Million tonnes
7	µm	Micro meter
8	et al.	et alli (and others)
9	Fig.	Figure
10	g	Gram
11	i.e.	Id est (that is)
12	mm	Millimeter
13	°C	Degree Celsius
14	p	Page
15	spp.	Species
16	viz.,	vi delictet (namely)
17	\$	Dollar
18	mL	Milliliter
19	L	Litre
20	HCl	Hydrochloric acid
21	NaOH	Sodium hydroxide
22	mg	Milligram
23	Kg	Kilogram
24	Hrs	Hours
25	Ha	Hectare
26	f. sp.	Forma specialis

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ABSTRACT

The investigation entitled “Epidemiology and management of Fusarium head blight of wheat” was conducted at the Department of Plant Pathology, CSK HPKV, Palampur and Research farm of Rice and Wheat Research Centre (RWRC), Malan during 2019-21. The average disease incidence and severity of FHB at different locations of Himachal Pradesh varied from 3.25 to 7.74 and 15.08 to 25.29 per cent during 2019-20 and 2020-21, respectively. Three *Fusarium* species viz., *Fusarium graminearum*, *F. compactum* and *F. avenaceum* were found associated with the disease but *F. graminearum* was more prevalent in all the surveyed locations of Himachal Pradesh. The temperature of 25°C and pH of 5.0 were found to be optimum for mycelial growth, sporulation and perithecia formation of the fungus. The seed and crop residue of maize, wheat and rice served as the primary source of inoculum for the disease development. All the weather parameters viz., temperature, relative humidity and rainfall contributed for the disease development as all of them individually and collectively showed positive correlations with disease severity. Out of 200 tested genotypes of wheat, 13 were found free from disease, 26 as resistant and 54 as moderately resistant to FHB under artificial inoculations. Under *in vitro* conditions, beejamrit, extracts of *Acorus calamus* (aqueous and alcoholic), *Trichoderma harzianum* (TH-5) and carbendazim 50 WP gave highest inhibition of *F. graminearum*. In a pot experiment, seed treatment and two foliar sprays of carbendazim 50 WP gave maximum reduction (71.85 & 77.16%) in disease incidence and severity, respectively. However, among non-chemical management components beejamrit, aqueous extract of *Acorus calamus* and *Trichoderma harzianum* (TH-5) gave maximum reduction in disease incidence and severity. An integrated treatment viz., seed treatment with carbendazim 50 WP (0.2 %) followed by three foliar sprays each of jeevamrit (1 %), aqueous extract of *Acorus calamus* (1 %) and carbendazim 50 WP (0.1 %) at 10 days interval was found to be most effective giving 57.26 and 61.56 per cent reduction in disease incidence and severity, respectively.

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1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is a dominant cereal crop and a staple food for millions of people around the world. It is believed to have originated from Southwest region of Asia. It is known as the "King of Cereals" as it can be cultivated under diverse climatic conditions and soils, than any other grain. It is also suitable for bread-making and is a constant part of the human diet. It is grown all over the world for its highly nutritious and useful grain, as one of the top three most produced crops, along with corn and rice. Wheat is a vital source of carbohydrates, is second only to rice as a source of calories (21%) and first as a source of protein (20%) in the diets of consumers. Its kernels contain protein (13%), starch (67%), arabinoxylan (8%), cellulose (2%) and beta glucan (0.8%). Its starch is widely used in the paper, cosmetics and pharmaceutical industries.

It is utilised in food industry to make cakes, bread, pasta, spaghetti, macaroni, biscuits, semovita, etc. Globally, it is cultivated over an area of 223.79 million hectares (mha), with production of 788.98 million tonnes (mt) per annum. India is the second largest producer of wheat after China, with an area of over 31.58 mha and a total production of 108.0 mt (Anonymous 2021). Wheat occupies around 0.33 mha area in Himachal Pradesh with production of around 0.63 mt (Anonymous 2020).

Wheat plays an essential role in the grain pool of the nation and is the leading source of food security in India. Regardless of proliferating wheat production in India, there is a constant pressure to feed the growing population on account of diminishing fertile land due to rapid industrialization and urbanization. It is anticipated that the world population will rise to 9 billion by the year 2050, which will require about 900 mt of wheat to feed the population (Panwar 2015).

Wheat in natural environments is predisposed to various biological, physiological and mechanical stresses that hamper its usual growth, development and production. One of the major constraints in improving wheat production is the appearance and pervasiveness of a number of diseases. The wheat crop is attacked by a large number of diseases caused by fungi, bacteria, viruses, nematodes and some deficiency syndromes, which result in a reduction in both quality and quantity. These

diseases plunder the bread basket of humans and influence the quantity and quality of wheat to a great extent.

Among fungal diseases of wheat, three types of rusts (stem, leaf, and stripe), loose smut, flag smut and karnal bunt are of significant importance. In addition to these, *Fusarium* head blight (FHB) is also a destructive disease of wheat in the humid and subhumid areas of the world. About 17 pathogens are found associated with FHB, of which *Fusarium graminearum* (Schwabe) is the principal pathogen responsible for this disease in many countries, including India. Other related species viz., *F. culmorum*, *F. avenaceum*, *F. oxysporum*, *F. moniliforme*, *F. poae* and *Microdochium nivale*, may also lead to a head blight complex (Wiese 1987). *Fusarium graminearum* was first described by Lewis Schwein in 1838, and the fungus was illustrated as the asexual (anamorph) phase of the teleomorph *Gibberella zeae* (Bolanos-Carriel 2018).

The occurrence of this disease results in the premature bleaching of one or more spikelets or entire heads (Wiese 1987). Bleaching generally starts from the middle of the head and progresses to all the spikelets and ultimately covers the entire head. A brown or purple-coloured peduncle can be seen immediately below the head (Parry et al. 1995). During prolonged wet weather, the fungus sporulates on infected spikelets and glumes, producing pink to salmon-orange spore masses. Bleached spikelets are sterile or contain kernels that are shrunken or appear chalky white or pink. Thus, the disease not only reduces the yield but also lowers the quality of grains. Infected grains harvested from an infected crop are also contaminated with various fungal mycotoxins, often making them unsuitable or unsafe for human consumption, animal feed, or malting purposes (Kimura et al. 2007; Wu and Munkvold 2008). Consumption of infected feedstuff by livestock may generate a variety of poor health effects involving feed refusal, diarrhea, reduced weight gain and emesis (Krska et al. 2001).

The infection by the FHB pathogen is favoured by low temperatures (20°C) and high relative humidity (100%), whereas wet conditions with temperatures around 25–30°C are congenial for further disease development. During epidemic years, FHB has been reported to cause yield losses from 10 to 70 per cent (Matthies and Buchenauer 2000). In India, FHB is though considered a disease of minor importance, but with changing climatic conditions due to the global warming, i.e. an increase in temperature and more frequent rainfall at the time of anthesis, it has attained the status

of an important disease in the country as well as in Himachal Pradesh. Moreover, it has been reported to cause yield losses between 15 to 29 per cent (Chaudhary et al. 1994) in the country, which are quite high.

Although different practices, such as sanitary measures, cultivating disease-free seed/ planting material, removal of diseased plants, cultivation of tolerant varieties, are employed for managing the disease, yet the use of chemical measures and resistant cultivars seems to be the most promising option. Registered fungicides can undoubtedly reduce the FHB infection (Suty and Mauler-Machnik 1997; Jones 2000; Matthies and Buchenauer 2000), but the residue concerns and environmental hazards may lead to their restricted use.

Although there is advancement in developing resistant cultivars through traditional breeding (Bai and Shaner 2004) and genetic modifications (Bushnell et al. 1998) in wheat, yet due to certain factors such as complexity of resistance, poor agronomic characters associated with resistance and low industrial quality, it is not feasible to transfer the resistance completely in cultivar, thus making them liable to infection. Likewise though, conventional tillage methods are quite effective in lowering FHB infection rates but are not compatible with the minimum tillage practise of soil conservation (Miller et al. 1998; Dill-Macky and Jones 2000). Keeping in view the incomplete control of the disease with individual management component and other deficiencies/ limitations involved in their use, an integrated management seems to be the most effective strategy to combat this disease.

In the country, most of the work conducted on FHB pertains to its occurrence/ prevalence, identification of associated *Fusarium* species, and some other pathological aspects. However, no systematic work has been done on this disease in India or Himachal Pradesh. Thus, it is pertinent to conduct elaborate studies on the epidemiology and management of FHB in the State. The present study is proposed to generate information on the following objectives:

1. To study the factors affecting pathogen and disease development
2. To evaluate wheat germplasm for identification of resistant sources
3. To evaluate integrated disease management components against *Fusarium* head blight

2. REVIEW OF LITERATURE

Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most devastating diseases of wheat in the world (Goswami and Kistler 2004). Besides yield and kernel quality losses, FHB has a wide range of consequences as the mycotoxins produced by the pathogen contaminate raw grain and processed wheat products (Edwards et al. 2009), thereby putting human and livestock health at risk. It has attained the status of an important disease in India as well as Himachal Pradesh as a result of changing climatic conditions caused by the global warming. The present investigation aims at systematic studies on the epidemiology, identification of resistance sources and management of FHB of wheat. The relevant literature pertaining to different aspects of the current study entitled “**Epidemiology and management of Fusarium head blight of wheat**” has been reviewed under the following headings:

2.1 Occurrence of the disease

2.2 Economic importance of the disease

2.3 Pathogen associated with the disease

2.4 Factors affecting pathogen growth and development

2.4.1 Effect of temperature

2.4.2 Effect of pH

2.5 Factors affecting disease development

2.5.1 Source of inoculum

2.5.2 Climatic factors

2.5.3 Host range of the pathogen

2.6. Identification of resistance sources

2.7. Management of the disease

2.7.1 Effect of different sowing dates

2.7.2 Evaluation of organic and natural preparations/ amendments

2.7.3 Evaluation of botanicals

2.7.4 Evaluation of biocontrol agents

2.7.5 Evaluation of fungicides

2.8 Integration of IDM components for management of the disease

2.1 Occurrence of the disease

FHB is widely distributed in wheat growing areas of the world. W.G. Smith in 1884 for the first time described *Fusarium* head blight as wheat scab in England (Stack 2003). He named the causal agent of wheat scab as *Fusarium culmorum*. The disease was later referred to as "Fusarium head blight" by Atanasoff (1920). Several epidemics of the disease have occurred in the past and its outbreaks have been reported in North America (Moschini and Fortugno 1996; Gilbert and Tekauz 2000), Eurasia (Parry et al. 1995; McCormick 2003), Australia (Burgess et al. 1987; Akinsanmi et al. 2004), South Africa (Scott et al. 1988; Kriel and Pretorius 2008) and Asia (Zhang et al. 2013).

Since the occurrence of the first epidemic of FHB in 1936, it has been a concern in some provinces of China (Chen et al. 2000). Although FHB is found sporadically in Bangladesh, yet the favourable climatic conditions can make the situation difficult (Duveiller 2004). Many Asian nations, including India largely follow rice-wheat system, in which FHB is more prevalent in the latter since the former serves as a host for inoculum buildup that might lead to an epidemic.

In India, *Fusarium* head blight of wheat was first reported in Arunachal Pradesh and Assam in 1973 (Roy 1974). Later, the disease was also reported in Amritsar and Gurdaspur districts of Punjab (Chahal et al. 1993). Singh and Aujla (1994) suggested that FHB can be a major stumbling block for wheat production in Punjab, as a serious epidemic of this disease occurred in Amritsar and Gurdaspur districts of Punjab in April-May 1990, particularly attacking variety HD 2329. Panwar et al. (2016) studied the distribution dynamics of *Fusarium* species causing FHB in wheat at different geographical locations in India and found *F. graminearum*, *F.*

pallidoroseum and *F. oxysporum* to be associated with the disease. However, *F. graminearum* was dominant at all the locations.

2.2 Economic importance of the disease

FHB can drastically reduce grain yields and in some cases it can cause up to 100 per cent yield loss in susceptible cultivars under favourable environmental conditions. Substantial yield loss occurs when shriveled or “tombstone” kernels are removed by the combine and moreover, their presence in the grain produce can significantly reduce the test weight (Bai and Shaner 1994). Although early infections by the pathogen are more relevant in terms of yield losses (Bushnell et al. 2003; Steffenson 2003), yet mycotoxin production ability also remains one of the major concerns in FHB (Del Ponte et al. 2007).

FHB epidemics in the United States and Canada in the mid-1990s resulted in significant losses in wheat production. In the United States alone, economic loss was predicted to be \$2500 million in wheat and \$400 million in barley, while in Canada it was estimated to the tune of \$220 million and \$300 million in wheat and barley, respectively (Windels 2000). The percentage of scabbed spikes normally varied from 30 to 50 per cent during severe epidemics, but it could reach 60 to 70 per cent in susceptible cultivars (Zhang 1998; Shi and Wang 1999). In Sichuan Province, the outbreak of FHB in 1996 caused a yield loss of around 78,000 metric tonnes (Li 1996). Matthies and Buchenauer (2000) observed that epidemic years of FHB might result into yield losses ranging between 10 to 70 per cent. Though yield losses of 5–10 per cent are usual in China owing to FHB, yet they can reach up to 100 per cent in epidemic years, affecting around 7 million hectares of wheat crop (Cheng et al. 2012).

In India, Chaudhary et al. (1994) reported yield losses of 15 to 29 per cent as a result of natural field infection of wheat by *F. graminearum* and *F. avenaceum*. Saharan et al. (2007) recorded moderate to high incidence of FHB in the bread wheat cultivar PBW 343 (10–50% infected heads) and the durum wheat cultivar PDW 274 (> 90% infected heads) in Gurdaspur district of Punjab.

Deoxynivalenol (DON) is the most common type of trichothecene found in the kernels produced by FHB-infected spikes, which can cause severe ailments in farm animals and human beings (Rotter et al. 1996). Ingestion of infected grains can induce

severe intestinal irritation in animals, leading to feed refusal in cattle, as well as a potentially deadly condition known as alimentary toxic aleukia (ATA) in humans and other mammals (Eriksen and Pettersson 2004).

2.3 Pathogen associated with the disease

Several pathogens have been found to be associated with head blight of wheat. During the 19th century, *Gibberella zeae* was considered to be the major pathogen in most FHB-affected areas of North America, while in Europe at the same time *Fusarium avenaceum*, *F. culmorum* and *F. nivale* were found associated with the disease (Atanasoff 1920). Many *Fusarium* species have been reported to cause FHB, but *F. graminearum* Schwabe (teleomorph *Gibberella zeae* Schwein Petch) is considered as the principal pathogen (O'Donnell et al. 2000; Saharan et al. 2003; Zeller et al. 2004). Other closely related species include *F. acuminatum* Ellis & Everhart, *F. equiseti* (Corda) Sacc., *F. culmorum* (Smith) Sacc., *F. avenaceum* (Fries) Sacc., *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* J. Sheld), *F. sporotrichioides* Sherb., *F. crookwellense* Burgess, Nelson & Toussoun, *F. oxysporum* Schlecht, *F. poae* (Peck) Wollenw and *Microdochium nivale* (Fries) Samuel & Hallett (Wiese 1987; Clear and Patrick 2000; Fernandez et al. 2001; Gilbert et al. 2001; Xue et al. 2002). *F. pseudograminearum* and *F. sacchari* were also found associated with FHB in China (Xu et al. 2015; Wang et al. 2015). In India, *F. graminearum*, *F. poae*, *F. avenaceum*, *F. compactum*, *F. verticillioides*, *F. oxysporum*, *F. pallidoroseum* (semitectum) and *Microdochium nivale* have been found associated with FHB (Roy 1974; Brahma and Singh 1985; Singh and Aujla 1994; Kaur et al. 1999; Mann and Nanda 1999; Saharan et al. 2003). Among all these, *Fusarium graminearum* is considered to be the most dominant causal organism of FHB (Panwar et al. 2016).

2.4 Factors affecting pathogen growth and development

2.4.1 Effect of temperature

Temperature is an important factor influencing the germination of spores of a pathogen and its further growth and development *in vitro* and *in vivo*. Tschanz et al. (1976) reported that the optimum temperature for perithecia production ranged from 15.0 to 28.5°C while, for ascospore production it ranged from 25 to 28°C (Sutton

1982). Rossi et al. (2002) studied the effect of constant temperature regimes on the sporulation of *Fusarium graminearum*, *F. avenaceum*, *F. culmorum* and *Microdochium nivale* on potato dextrose agar media and found that within each species, different isolates revealed comparable sporulation-temperature associations. Among them, *F. avenaceum* produced the largest number of spores, followed by *M. nivale*, *F. graminearum* and *F. culmorum*. However, the optimum temperature for macroconidia production was 32°C for *F. graminearum*, 28°C for *F. avenaceum* and 26°C for *M. nivale*.

Brennan et al. (2003) studied the effect of temperature on *in vitro* growth rate of a European *Fusarium* collection and found that the optimum temperature for the growth of *F. graminearum*, *F. culmorum* and *F. poae*, regardless of geographic origin, was 25°C, while it was 20°C for *F. avenaceum* and *M. nivale*. *Fusarium graminearum*, *F. culmorum* and *F. poae* grew faster between 10 and 25°C, while *F. avenaceum* and *M. nivale* grew faster between 10 and 20°C. Dufault et al. (2006) observed that perithecia were formed at temperature ranging from 12 to 28°C in the laboratory and perithecial maturation occurred best at 20–24°C.

Osborne and Stein (2007) observed that *Fusarium graminearum* could grow over a broad range of temperature up to 30°C and usually found in the warmer areas of the world. The minimum soil temperature for perithecia formation varied from 6 to 10°C, with an ideal temperature range of 15 to 20°C (Pereyra et al. 2004; Gilbert et al. 2008). Hudec and Machova (2010) studied the effect of temperature on *in vitro* growth rate of *Fusarium* species and reported that *F. avenaceum*, *F. culmorum*, *F. graminearum* and *F. poae* grew at faster rate at 25°C, while *M. nivale* at 15°C.

Manstretta and Rossi (2016) studied the effect of temperature on perithecia formation in the laboratory and revealed that these were produced at the temperatures between 5 and 30°C with optimum being 21.7°C. However, no perithecia were formed at 35 and 40°C and just a few produced at 5°C. Nazari et al. (2018) observed that the optimal temperatures for colony growth and nivalenol (NIV) production by *Fusarium poae* were 24.7 and 27.5°C, respectively.

2.4.2 Effect of pH

Different plant pathogens are known to have specific requirement of pH levels for their growth, development and sporulation. Sharma et al. (2005) studied the effect of various pH levels (4.5 to 8.0) on the growth and sporulation of *Fusarium oxysporum* f. sp. *lini* and observed that the pathogen showed the highest growth and sporulation at pH 5.5. Tyagi and Paudel (2014) conducted *in-vitro* studies to check the effect of pH on the growth and sporulation of *Fusarium oxysporum* and observed that pH of 6.0 was optimum for the growth as well as sporulation of the fungus. However, the chlamydospore sporulation occurred best at pH level 4.5 and its further increase resulted in retarding effect on the growth and sporulation of the fungus.

Cruz et al. (2019) studied the effect of five pH levels (4.0 to 8.0) and four incubation temperatures (15, 20, 25 and 30°C) on the growth of 14 isolates of *Fusarium oxysporum* and found that all the isolates showed the highest radial growth at pH 6.0 incubated at 25°C. Pal et al. (2019) conducted an *in vitro* experiment to find out the suitable pH for the growth and sporulation of *Fusarium oxysporum* f. sp. *lini* and observed that the maximum growth (86.33 mm) of the fungus occurred at pH 5.5 while, the highest sporulation of 8.2×10^6 per mL obtained after 9 days of incubation at $25 \pm 2^\circ\text{C}$. Poorvasandhya et al. (2020) carried out an experiment to study the effect of pH on mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *udum* and found that its most suitable levels for the former and latter were 6.0 and 7.0, respectively.

2.5 Factors affecting the disease development

2.5.1 Source of inoculum

The source of inoculum (primary) plays a major role in the initiation and further buildup of a disease in a crop. The pathogen, *Fusarium graminearum* can be found in old maize stalks as well as ears, stubbles and debris of wheat, barley and other cereal crops (Gordon 1959; Burgess and Griffin 1968; Warren and Kommedahl 1973). Burgess and Griffin (1968) obtained *Fusarium graminearum* from the colonised wheat straw after 104 weeks of its burial in Australian field soils. However, the survival period of the pathogen was decreased to 56 weeks or less by frequent wetting of the soil. In Minnesota, Warren and Kommedahl (1973) noted that the pathogen overwintered on wheat straw, although the proportion of straw colonisation

decreased from 40 to 24 per cent during the winter and *F. graminearum* lived longer in the tissues that were resistant to degradation in general. Windels and Kommedahl (1974) recovered the pathogen from only 30 per cent of soil samples from maize fields in Minnesota, which indicated that the soil was not the major source of inoculum. Wearing and Burgess (1977) recovered the fungus on dilution plates of soils from wheat-fields of semi-arid regions of Australia and reported that it grew largely from minute pieces of organic debris, presumably from wheat.

F. graminearum is primarily found in host debris, although it can also be found in soil, seed and different susceptible (Sutton 1982). The crop residues play an important role in the perennation of *F. graminearum*, since it survives in the form of mycelia or immature perithecia on infected wheat spikelets, maize ear residues or on maize and wheat stubbles (Teich and Nelson 1984). Among the crop residues, cereal grains, as well as maize stem nodes and grains are the most prolific source of inoculum (Khongla and Sutton 1988; Bai and Shaner 1994).

Katan et al. (1997) observed that the macroconidia were transported from the soil or infected leaves to flowering spikes by rain-splashes which played a predominant role in dispersal of the pathogen. Markell and Francl (2003) noted that the sexual cycle of *F. graminearum* started from overwintered perithecia (small, bluish-black, round bodies on the crop residues) which were found to retain their viability up to 16 months and were abundant in spring, as the warm moist weather conditions favoured their maturation. Pirgozliev et al. (2003) concluded that FHB infection on wheat was 15 per cent more when it was sown after maize crop as compared to only 4 per cent when it was grown following oats or alfalfa. Gilbert and Fernando (2004) stated that Fusarium damaged kernels (FDK) were not the source of inoculum and did not cause the infection. Bai and Shaner (2004) reported that though macroconidia, chlamydospores and hyphal fragments could all serve as source of inoculum yet ascospores were the primary source of inoculum that caused infection.

The FHB pathogen is capable of surviving as a saprophyte on crop debris and contributes to inoculum for infection of wheat plants in the same field in which the residue is present and more distant fields (Keller 2011). Leplat et al. (2013) reported maize to be the more important source of inoculum in locations where both small

grain cereals and maize were grown because the debris of latter lasted longer than the former. Moreover, *Fusarium* survival was aided by nutrient-rich maize residues. Maize as the preceding crop is linked with the highest risk of FHB because it is an excellent host of *Fusarium* spp. and produces a considerable amount of residues (Dill-Macky and Jones 2000; Tillmann et al. 2017). Njeru et al. (2014) found that *Fusarium oxysporum* and *F. proliferatum* were the most common species recovered from the soil, whereas *F. chlamydosporum* and *F. graminearum* were isolated from crop residues. The intensity of *Fusarium* spp. in crop residues and soil differed significantly throughout agroecological zones of North County, Kenya.

2.5.2 Climatic factors

Climatic factors viz., temperature, relative humidity and rainfall are known to play a significant role in the occurrence and development of crop diseases. Anderson (1948) observed that the infection of wheat ears by *F. graminearum* occurred more frequently at 25°C. Sutton (1982) noted that FHB development and establishment occurred when warm temperatures were accompanied by a period of 92-94 per cent relative humidity during anthesis in wheat crop. Shaner (2003) also found that FHB development was favoured by high rainfall, high relative humidity, warm temperatures and reduced tillage and the presence of crop residue on the soil surface.

Champeil et al. (2004) reported that the head and grain infection occurred when the temperature during flowering was more than 20°C and the humidity exceeded 85-90 per cent for at least 24-40 hours. Saharan et al. (2004) observed that FHB was more prevalent in Northern India because of warm and humid weather during March and April. Bateman (2005) reported that FHB developed more likely when the pathogen was present at the soil level and the weather was warm and moist, with frequent rains between blooming and kernel filling phase.

Osborne and Stein (2007) observed that the infection of *F. graminearum* could take place over a broad range of temperature and humid conditions. Rossi et al. (2001) studied the effect of humidity and temperature on the infection of wheat spikes by some causal organisms of FHB and found that the infection rate was optimal at 26.5°C for *F. culmorum* and 28-29°C for *F. graminearum* and *F. avenaceum*. Weather factors such as moderately high temperature (15 to 30°C), frequent precipitation, prolonged

periods (48–72 hrs) of high moisture and occurrence of air currents all favoured FHB infection (Lenc 2015).

2.5.3. Host range of the pathogen

Host range of a plant pathogen plays an important role in its survival in the nature. FHB is caused by a large number of *Fusarium* species viz., *Fusarium graminearum*, *F. poae*, *F. culmorum*, *F. sporotrichoides*, *F. avenaceum* and *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme*) affecting gramineous hosts (Osborne and Stein 2007). *Fusarium* species (teleomorph *Gibberella*) are distributed worldwide, causing several diseases such as foot rot, seedling blight, *Gibberella* stalk and ear rot of maize and *Gibberella* seedling blight and head scab of small grain (wheat, oats, barley, rye and triticale) cereals (Popovski and Celar 2012; Shude et al. 2020). Members of the *Fusarium graminearum* species complex (FGSC) are among the most important fungal plant pathogens in the world, causing significant diseases in major cereal crops such as wheat, maize, rice and barley (Dean et al. 2012; Van der Lee et al. 2015).

2.6. Identification of resistance sources

Identification of resistant sources as varieties for direct cultivation or resistant genetic stocks for developing improved cultivars, is the only most effective, economic and practical method of disease control.

Khalil et al. (2000) evaluated 19 cultivars and 21 genotypes of wheat against FHB and found that five each of cultivars (Kalyan Sona, Innia 66, Kari, Barkat and Pavan) and genotypes (NK5, BKT/Bulbul, TP2, KRL-1-4, and O4) to be resistant to the disease. Muthomi et al. (2007) evaluated eight cultivars of wheat for their resistance to FHB under green house conditions and found Njoro Bw1 and Njoro Bw2 to be most promising. Zhang et al. (2008) observed that out of 1035 accessions of wheat screened for resistance to FHB, 73 were resistant to it.

Chrpova et al. (2013) evaluated 35 spring wheat varieties for resistance to FHB under field conditions and found that four genotypes (Tabor, May Emmer, Rudico and Weisser Sommer) of *T. dicoccum*, one genotype (Ruzyne), of *T. spelta*, a common wheat landrace (Cervena perla) and a bread wheat variety (Vanek) were

moderately resistant to the disease. Villiers (2014) screened 13 entries of wheat for FHB resistance with SST 825 and Sumai 3 as susceptible and resistant control, respectively and found one entry to be resistant while three to be moderately resistant.

Espinoza et al. (2019) screened wheat germplasm against FHB and found that 8 varieties were resistant while, 12 were moderately resistant. Huang et al. (2019) tested three new wheat lines L958, L962 and L987 for resistance to FHB and found that all the lines were moderately resistant to the disease. Shi et al. (2020) screened 205 Chinese wheat cultivars for resistance to FHB during 2017 and 2018 and found that more than 76 per cent cultivars were susceptible to FHB in both the years. However, some cultivars like Tianminxiaoyan369, Yangao168 and Zhongyu1526 showed resistant reaction in 2017 and moderately resistant reaction in 2018. While, cultivar Zhongwo9 showed moderately resistant reaction in 2017 and resistant reaction in 2018. Eight cultivars (Minfeng266, Cunmai608, Junda159, Zhengmai162, Fengmai12, LS4607, Pumai1165 and Deyan1603) were moderately resistant to FHB during both the years.

Bentivenga et al. (2021) screened 16 durum wheat cultivars against FHB and found that most of the cultivars were susceptible to very susceptible with the exception of Dylan, Tiziana and Dupri which were moderately susceptible to the disease. Sakr and Al-Attar (2021) evaluated six wheat cultivars against four *Fusarium* species of head blight under field conditions during 2020 and 2021 and found two cultivars viz., Bohoth10 and Cham4 to be moderately resistant to the disease.

2.7. Disease Management

2.7.1 Effect of different sowing dates

Sowing date is one of the important elements of crop management as it indirectly determines whether the infection of a crop by a pathogen will take place or there will be an escape. When the crop is sown at such a time that the anthesis period coincides with the spore production or release, then severe attacks by the pathogens are more likely to take place (Strange and Smith 1971; Lacey et al. 1999). Arthur (1891) reported that early sown wheat varieties were more resistant to FHB than the varieties that mature late. Parikka et al. (2008) observed that late-maturing lines of oat were more susceptible to FHB than the early-maturing ones. Subedi et al. (2007)

found that in both the heavy and light textured soils, planting date exhibited a consistent stronger influence on the incidence and severity of FHB.

2.7.2 Evaluation of organic and natural preparations/ amendments

Akinnuoye-Adelabu et al. (2019) examined the effect of redworm extracts (mucus and vermiwash) against *Fusarium graminearum* D52 under *in vitro* conditions and found that pure mucus extract showed the highest mycelial growth inhibition ($19.46 \pm 5.8\%$), followed by 14-days old vermiwash ($13.77 \pm 2.4\%$) and 7-days old vermiwash ($10.66 \pm 0.7\%$).

Pathania et al. (2019) evaluated five organic preparations against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions and found that cow urine at 10 per cent concentration showed the maximum mycelial inhibition (72.9%), followed by panchgavya, beejamrit and vermiwash with 62.9, 59.2 and 38.5 per cent inhibition, respectively. Kaur (2019) evaluated five organic inputs against *Fusarium solani* f. sp. *pisi* and found that jeevamrit at 8 per cent concentration showed 100 per cent mycelial inhibition followed by panchgavya (62.7%), beejamrit (57.4%), *Eupatorium adenophorum* extract (31.1%) and himsol (25.2%) all at 10 per cent concentration. Rana et al. (2020) tested six organic formulations against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions and found that at 10 per cent concentration, panchgavya showed 92.22 per cent mycelial inhibition of the pathogen followed by biosol, cow urine, vermiwash and beejamrit with 72.22, 64.44, 61.11 and 59.63 per cent mycelial inhibition, respectively.

2.7.3 Evaluation of botanicals

Use of botanicals for the management of plant diseases is comparatively a new ecofriendly approach. Salhi et al. (2017) studied the antifungal properties of aqueous extracts of four plant species against *Fusarium graminearum* and *F. sporotrichioides* and found that *Asphodelus tenuifolius* extract at 10 and 20 per cent concentration resulted in 56.89 and 60.34 per cent mycelial inhibition of *F. graminearum*, respectively. Whereas, *Euphorbia guyoniana* at same concentrations resulted in 50.87 and 65.87 per cent mycelial inhibition of *F. sporotrichioides*.

Seepe et al. (2021) evaluated leaf extracts of *Withania somnifera* (L.) Dunal and *Combretum erythrophyllum* (Burch.) Sond. for their antifungal activity against *Fusarium* pathogens (*F. graminearum*, *F. oxysporum*, *F. subglutinans*, *F. semitectum*, *F. verticilloides*, *F. proliferatum*, *F. solani* and *F. chlamydosporum*) and found that they exhibited good antifungal activity against one or more of the tested *Fusarium* species with minimum inhibitory concentrations (MIC) less than 1.0 mg/ mL. Dinev et al. (2021) studied the antifungal activity of the methanolic extracts of four plants against *Fusarium graminearum*, *F. oxysporum*, *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *A. niger*, *A. carbonarius*, *Penicillium chrysogenum* and *Alternaria alternata* and noted that *Acorus calamus* extract showed the highest antimicrobial activity against eight fungal pathogens, followed by *Chlorella vulgaris*, *Lemna minuta* and *Scenedesmus dimorphus* extracts, which provided inhibition of only two to three pathogens.

Hasan et al. (2005) evaluated ten plant extracts for their efficacy against seed-borne fungi of wheat under *in vitro* conditions and found that seed treatment with alcoholic extracts of garlic (*Allium sativum*) and neem (*Azadirachta indica*) completely controlled the occurrence of *Fusarium* spp., *Bipolaris sorokiniana*, *Rhizopus* spp., *Penicillium* spp., and *Aspergillus* spp. Siva et al. (2008) tested the antifungal effect of four plant extracts against *Fusarium oxysporum* f. sp. *melongenae* under *in vitro* conditions and found that *Vitex negundo* at 10 per cent concentration showed the highest inhibitory effect (82%), followed by *Sapindus emarginatus* and *Adhatoda vasica*, with 74 and 72 per cent inhibition, respectively.

Rawal et al. (2015) studied the antifungal activity of *Acorus calamus* against *Fusarium oxysporum* f. sp. *lycopersici* by paper disc diffusion method and found that its acetone extract at 1000 mg/ mL concentration was most effective giving inhibition zone of 29.5 mm followed by ethanol and aqueous extracts, with inhibition zones of 19.6 and 15.1 mm, respectively. Rawal et al. (2016) evaluated acetone extracts of four medicinal plants against *Fusarium oxysporum* f. sp. *lycopersici* and observed that *Arctium lappa* extract was the most potent with 85.52 per cent mycelial inhibition, followed by *Acorus calamus*, *Origanum vulgare* and *Thymus serpyllum* with 66.89, 36.40 and 40.63 per cent inhibition, respectively.

Nefzi et al. (2016) evaluated organic and aqueous extracts of fruits, leaves and stems of *Withania somnifera* L., for antifungal activity against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) and found that aqueous extract of fruits at 2 per cent concentration showed the highest antifungal potential, with 56.27 per cent reduction in growth as compared to stem and leaf extracts which gave 52 and 45.34 per cent reduction at 3 per cent concentration, respectively. Among the three organic extracts, butanolic stem extract at 4 per cent concentration showed the highest antifungal activity with 62.03 per cent reduction in the radial growth of the pathogen.

2.7.4 Evaluation of biocontrol agents

The biocontrol agents have a great potential in the management of many soil borne and foliar diseases. They colonize the crop residue present in the soil and through their antagonistic effect control the pathogen(s) harbouring the substrate. Nourozian et al. (2006) studied the antagonistic effect of bacterial strains such as *Bacillus subtilis* strains 53 and 71, *Streptomyces* spp. strain 3 and *Pseudomonas fluorescens* biov1 strain 32 against *Fusarium graminearum* and observed that the cell free and volatile metabolites of these reduced the mycelial growth of the pathogen to the tune of 37.00 - 97.00 per cent. Popiel et al. (2008) examined the effect of 92 antagonist fungal isolates (belonging to 29 species) against three toxigenic *Fusarium* species (*Fusarium graminearum*, *F. avenaceum* and *F. culmorum*) of cereals and found *Trichoderma* spp. to be highly effective. Shi et al. (2014) isolated 32 bacterial strains from peanut shells and investigated their activity against *F. graminearum* by dual-culture plate and tip-culture assays and found that twenty strains gave 41.41 to 54.55 per cent growth inhibition of the pathogen by the first and 92.70 to 100 per cent through the second method. Comby et al. (2017) evaluated 86 strains isolated from the inner tissues of wheat plants against *F. graminearum* and *F. culmorum* and found 22 of them to be effective giving 30–51 and 15–53 per cent inhibition of former and latter, respectively. Blaszczyk et al. (2017) evaluated 24 isolates of *Trichoderma* spp. against five species of *Fusarium* (*F. temperatum*, *F. avenaceum*, *F. graminearum*, *F. culmorum* and *F. cerealis*) and found that all the isolates were potent in influencing the mycelial growth of at least four of them in dual-culture bioassay. They also concluded that out of all the screened *Trichoderma* isolates, *T. atroviride* AN240 was most effective in suppressing mycotoxins (69–100% toxin reduction) production by

all the five *Fusarium* species. Gupta (2019) assessed various bioagents against *F. graminearum* and found that *T. harzianum* (TH-5) was most effective as it resulted in 40.33 per cent mycelial inhibition in dual culture whereas, *T. harzianum* (TH-11) was least effective with 20.60 per cent mycelial inhibition.

Fernandez (1992) reported that inoculation of oat and wheat straw with *Trichoderma harzianum* resulted in reduced incidence of *Fusarium graminearum*, *Cochliobolus sativus* and other *Fusarium* species as a result of colonization of substrate by the bioagent. Czaban et al. (2004) reported that the inoculation of wheat seeds with two strains of *Pseudomonas fluorescens* (III107 and II21) and two strains of *Bacillus mycoides* (JC192 and K184) isolated from winter wheat roots as well as with one strain of *P. fluorescens* (ID13) isolated from oat roots reduced the infection of *Fusarium culmorum* on winter wheat in a pot experiment. Sarrocco et al. (2013) tested *Trichoderma gamsii* 6085 against FHB of wheat under field conditions and found that its application on spikes resulted in reduction of FHB severity by 57 per cent.

Wheat seed treatment with bacterial bioagents fluorescent pseudomonads gave promising results in controlling *Fusarium* seedling blight caused by *F. graminearum*, *Microdochium nivale* and *F. culmorum* under glasshouse and field conditions (Bello et al. 2002; Johansson et al. 2003). Riungu et al. (2008) evaluated different antagonists viz., *Epicoccum* spp., *Alternaria* spp., *Trichoderma* spp. and *Bacillus* spp. along with fungicides Folicur and copper oxychloride against FHB pathogen of wheat under *in vitro* as well as greenhouse conditions and found that Folicur and copper oxychloride completely inhibited the growth of the pathogen *in vitro*, while among bioagents *Trichoderma* spp. showed highest reduction in colony growth. Under greenhouse conditions, Folicur gave the maximum reduction in disease severity (47%) followed by copper oxychloride (36%). Hasan et al. (2012) studied the antagonistic activity of *Trichoderma harzianum* on a range of seed borne fungal pathogens of wheat and found that it inhibited the radial growth of *F. graminearum* by 43.33 per cent. The antagonist was also found effective under field conditions as it increased seedlings emergence (plant stand) by 15.93% over that the control. Panwar et al. (2014) evaluated *Trichoderma viride* isolate (Tv) and *T. harzianum* isolates (Th-M and Th-P) against FHB pathogen and found that *T. harzianum* (Th-M) significantly

reduced the mycelial growth of all the isolates of *F. graminearum* followed by *T. viride* (Tv) isolate. Their combined foliar application also resulted in reduced disease severity. Mahmoud (2016) obtained inhibition rate of 51, 49, 65, 63, 60, 46 and 48 per cent in the mycelial growth of *F. graminearum* (F.g.8) with seven isolates of *T. harzianum* while, 45, 55, 46, 43 and 42 per cent with five isolates of *Bacillus subtilis*, respectively. *T. harzianum* isolate (T.h.3) and *B. subtilis* isolate (B.s.2) showed 55.0 and 36.7 percent reduction in head blight severity under greenhouse conditions.

Ahmed et al. (2013) evaluated 53 fungal isolates recovered from wheat rhizosphere for their antagonistic potential against the damping off pathogens and found that only 11 fungal isolates showed moderate to high antagonistic effect against all the tested pathogenic fungi. Out of these, two *Trichoderma harzianum* isolates were quite effective in inhibiting the mycelial growth of pathogens and soil treatment with them significantly reduced the incidence of damping-off in Banyswif-1 and Giza-168 cultivars under greenhouse conditions. Kaur (2019) evaluated five fungal bioagents and one PGPR (*Pseudomonas fluorescens*) against *Fusarium solani* f. sp. *pisi* and found that SMA-5 strain of *Trichoderma harzianum* was the best with mycelial inhibition of 76.0 per cent followed by *T. viride* (73.8%), DMA-8 strain of *T. harzianum* (61.9%), JMA-11 strain of *T. koningii* (50.0%) and *P. fluorescens* (36.3%). Bouanaka et al. (2021) tested the biocontrol potential of 15 isolates of *Trichoderma* (T1 to T15) against four strains of *Fusarium culmorum* (FC11, FC2, FC4 and FC20) and found that T9, T12 and T14 resulted in 81.81, 77.27 and 80.68 per cent mycelial inhibition of FC2 strain, respectively. While in tube and pot experiments, T14 decreased the disease severity of *F. culmorum* to the tune of 50.00 and 63.63 per cent, respectively.

2.7.5 Evaluation of fungicides

Chemical control is one of the most effective and oldest methods of disease management both in aerial and soil borne diseases of plants. Boshoff et al. (1999) evaluated six fungicides against *Fusarium graminearum* and *F. crookwellense* and found that Flusilazole and prochloraz gave significant growth inhibition of *F. graminearum* with EC50 values of 0.353 and 0.027, respectively. Whereas, prochloraz and tebuconazole were found effective against *F. crookwellense* at EC50

values of 1.126 and 0.337, respectively. Mullenborn et al. (2008) reported the high sensitivity of different *Fusarium* spp. (*F. graminearum*, *F. culmorum* and *F. poae*) to tebuconazole and prothioconazole as compared to fluoxastrobin and azoxystrobin under laboratory conditions. Ivic et al. (2011) studied the effect of five fungicides on different isolates of *Fusarium graminearum*, *F. avenaceum* and *F. verticillioides* and found that prochloraz showed maximum growth inhibition of all the three species while, metaconazole showed better results as compared to tebuconazole and carbendazim. Avozani et al. (2014) evaluated the effect of three fungicide groups viz., Tubulin synthesis inhibitors, Demethylation inhibitor (DMI), Quinine outside inhibitors (QoI) and two readymade mixtures on mycelial growth or conidial germination of 5 isolates of *F. graminearum* and found that DMI's showed lower IC50 values as compared to the QoI's. Azoxystrobin was the most potent fungicide to inhibit spore germination, whereas DMI's provided the increased efficiency in the mycelial growth inhibition. Gupta (2019) evaluated various fungicides *in vitro* against *Fusarium graminearum* and found that Vitavax Power and Bavistin gave complete mycelial inhibition even at 50 ppm concentration, whereas tilt at same concentration was least effective with 55.74 per cent mycelial inhibition.

Boyacioglu et al. (1992) found that application of propiconazole and triadimefon fungicides at the stage of anthesis resulted in 34-79 and 39-61 per cent reduction in FHB infection and deoxynivalenol (DON) levels, respectively. Whereas, thiabendazole was effective in preventing the production of DON but ineffective in preventing FHB infection at all the stages of application. Cromeey et al. (2001) found that, two applications of tebuconazole at anthesis stage in a field trial resulted in 90 per cent reduction in FHB incidence and 14 per cent increase in the yield while, application of carbendazim, azoxystrobin and tebuconazole at full ear appearance or mid anthesis stage in another trial, resulted in reduced FHB incidence and increased grain weight. Pirgozliev et al. (2003) applied metaconazole and azoxystrobin with conidia of *Fusarium graminearum* and *F. culmorum* and found that the former showed high antimicrobial activity against both the pathogens and reduced FHB severity as compared to the latter. Haidukowski et al. (2005) evaluated different fungicides against FHB and found that application of cyproconazole plus prochloraz (Tiptor S) and a mixture of tebuconazole (Horizon 250 EW) plus azoxystrobin

(Amistar 250 SC) significantly reduced the FHB severity (25 to 77 %) and DON content (32 to 89 %) in the grain as compared to the inoculated control. Bagga (2008) evaluated six fungicides against FHB and found that Amistar reduced disease incidence and severity by 34.8 and 44.0 per cent, respectively in durum wheat cv. PDW 274. While it resulted in 74.4 and 89.3 per cent reduction in disease incidence and severity, respectively in bread wheat cv. PBW 502. Islam et al. (2015) evaluated three fungicides (Vitalax 200, Bavistin and Captan) for the control of seed borne fungi of wheat and found that seed treatment with Vitalax 200 was found highly effective as compared to others. Machado et al. (2017) reported triazoles (tebuconazole, prothioconazole and metconazole) and benzimidazoles to be the most effective fungicides for controlling FHB of wheat.

Jones (2000) reported that the application of fludioxonil at the anthesis stage reduced FHB incidence, severity and deoxynivalenol toxin concentration, but increased the percentage of plump kernels and yield. He also found that *F. graminearum* isolates were sensitive to benomyl, tebuconazole, fludioxonil and mancozeb at concentrations of 3.5×10^{-1} , 9.9×10^{-1} , 7.4×10^{-3} and 7.3 mg a.i./ L, respectively under laboratory conditions. Masiello et al. (2019) tested the sensitivity of *Fusarium graminearum*, *F. verticillioides*, *F. proliferatum* and *A. flavus* to eleven fungicides *in vitro* and observed that demethylation inhibitors (DMI) performed best under *in vitro*, followed by thiophanate-methyl and folpet. In field trials, prothioconazole and thiophanate-methyl were potent enough to reduce *F. graminearum* (52 % and 48 %) and *F. proliferatum* contamination (44 % and 27 %).

2.8 Integration of IDM components under field conditions

Fusarium graminearum is an ubiquitous fungus that is found on many hosts, making it very hard to control with a single strategy (Bai and Shaner 1994). The cultivation of resistant cultivars along with the use of proper management strategies is the best approach to manage FHB in wheat. McMullen et al. (2008) evaluated the efficacy of different integrated approaches to control FHB of wheat and found that the use of crop rotation, crop rotation + tolerant cultivar, crop rotation + tolerant cultivar + fungicide application provided 50, 80 and 92 per cent reduction in disease infection, respectively.

Blandino et al. (2012) conducted a study on integrated strategies for FHB control and deoxynivalenol contamination in winter wheat and found that the amalgamation of ploughing, a moderately resistant variety and triazole fungicide application at heading showed 97 per cent reduction of DON in FHB-contaminated grains. Wegulo et al. (2011) reported that fungicide (prothioconazole + tebuconazole) efficacy in reducing FHB and DON was higher in moderately resistant than in susceptible winter wheat cultivars. Willyerd et al. (2012) found that a combination of fungicide (prothioconazole + tebuconazole) application and moderately resistant cultivars provided the best control of FHB. Mengesha et al. (2021) found that the lowest severity (7.70 %), AUDPC (130.26 %-day) and the highest grain yield (6.68 t/ ha) were recorded at Bonke while, the highest disease severity (36.46 %), AUDPC (404.78 %-day) and the lowest grain yield (2.42 t/ ha) were recorded at North Ari. They further concluded that the integration of moderately resistant cultivars and fungicide with appropriate spray frequencies right from the onset of the disease reduced disease pressure and increased grain yield.

3. MATERIALS AND METHODS

The present investigation on “**Epidemiology and management of Fusarium head blight of wheat**” was carried out at Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur. All the *in vitro* experiments were conducted in PG laboratory of the Department of Plant Pathology, CSKHPKV, Palampur, whereas all the field experiments were conducted at the Research farm of Rice and Wheat Research Centre (RWRC), Malan during the period 2019-2021. The details of materials and methodologies used during the course of study are as given below.

3.1 Materials

During the course of the present study, the following materials were used:-.

3.1.1 Glasswares

The standard borosil glasswares were used to conduct various laboratory experiments. The glasswares used consisted of test tubes, petri plates, flasks (50, 100, 250, 500 and 1000 mL), funnels, beakers (100, 250 and 500 mL) and measuring cylinders (100 and 500 mL).

Sterilization of glasswares

These glasswares were dipped overnight in chromic acid solution prepared by dissolving 60 g of potassium dichromate ($K_2Cr_2O_7$) in approximately 150 mL of warm distilled water and then slowly adding concentrated sulfuric acid (H_2SO_4) to produce a total volume of one liter. These were further cleaned by washing with the detergent followed by rinsing with tap water. All the glasswares were sterilized either by dry heat sterilization. The dry heat sterilization was carried out at 180°C for 2 hours in hot air oven.

3.1.2 Equipment and apparatus

The equipment and apparatus used during the research work comprised of inoculation needle, forcep, surgical blade, cork-borer, spirit lamp, laminar air flow chamber, BOD incubator, hot air oven, compound microscope, autoclave,

haemocytometer, refrigerator, electric balance etc. Inoculation needle, forcep and blade were used for the isolation of the pathogen from the diseased plant sample. Inoculation needle was disinfected by dipping it in spirit and heating it over a flame 2-3 times until it was red hot and then it was allowed to cool down. In the same way, the forceps and cork-borer were also sterilized before the use. An electronic or digital balance was used for weighing different chemicals (including fungicides), botanicals, biocontrol agents and organic preparations. An autoclave was used to sterilize media. While, an oven was used to sterilize glasswares. Fungal cultures were incubated in BOD incubator(s). Cultures were preserved and maintained in a refrigerator. Haemocytometer (Neubauer) was used to count the spore concentration of the pathogen(s). All the pathological work was performed in a laminar air flow cabinet. Its working table was disinfected by swabbing the surface with spirit-soaked cotton and exposing it to UV light for 20 minutes. A pH meter (Eutech Instruments) was used to obtain different pH levels of medium under *in vitro* studies.

3.1.3 Culture medium

Potato Dextrose Agar (PDA) medium was used for the isolation and culturing of the pathogen(s). For the preparation of medium, 200 g peeled potatoes were cut into small pieces and boiled in 500 mL of distilled water in a steel pot. The extract was strained through a piece of muslin cloth and then 20 g dextrose was added to it. After that, agar-agar (20 g) was added in potato dextrose solution and stirred with the glass rod to avoid the formation of clumps and then it was allowed to boil. The final volume of the medium was made to 1000 mL by adding distilled water. This medium (PDA) was then poured in flasks, plugged with non-absorbent cotton plugs and sterilized in an autoclave at 15 lbs psi pressure and 121.6°C temperature for 20 minutes.

Preparation of slants

PDA slants were prepared by pouring approximately 3 mL medium in each culture tube, plugged with non-absorbent cotton plugs and sterilized in an autoclave at 15 lbs psi pressure and 121.6°C temperature for 20 minutes. After sterilization, tubes were kept in a slanting position with the support of a wooden rod and allowed to solidify. Slants were enclosed in a polythene bags and stored in a refrigerator.

3.1.4 Disease samples

The infected earheads showing typical symptoms of *Fusarium* head blight were collected in paper bags from the wheat crop grown in the farmers' fields and brought to the laboratory for further studies. Soil samples, seed and stubbles of cereal crops (maize, wheat and rice) from the infected fields were also collected in brown paper bags at the end of the *Kharif*, *Rabi* and *Kharif* season, respectively. These were kept/maintained at room temperature before use.

3.1.5 Organic and natural preparations/ amendments

Freshly prepared organic preparations viz., Vermiwash, Beejamrit, Jeevamrit, Brahmastra, Tamarlassi, Eupatorium plant extract and Biosol were procured from the Department of Organic and Natural farming. Neem and Mustard cakes were procured from market and preserved at room temperature.

3.1.6 Botanicals

Leaves of botanicals viz., *Azadirachta indica* (Neem), *Melia azedarach* (Darek), *Eucalyptus tereticornis* (Eucalyptus), *Eupatorium adenophorum* (Crofton weed), *Ranunculus muricatus* (Spinyfruit buttercup), *Euphorbia royleana* (Royle's spurge), *Vitex negundo* (Chinese chaste tree), *Polygonum hydropiper* (Water pepper), *Withania somnifera* (Winter cherry), *Lantana camara* (Lantana) and rhizomes of *Acorus calamus* (Sweet flag) were collected from adjoining areas of Palampur.

3.1.7 Bio-control agents

The fresh cultures of four fungal bio-agents viz., *Trichoderma harzianum* (TH-5), *T. viride* (Tv), *T. koningii* (DMA-8) and *T. koningii* (JMA-11)) and one bacterial bioagent viz., *Pseudomonas fluorescens* available in the Department of Plant Pathology were tested for their antagonistic effect against the test pathogen. In addition to these six fungal bioagents were isolated from the wheat rhizosphere and evaluated against *Fusarium graminearum*.

3.1.8 Seed material

Seed of wheat variety HPW 89 was used for performing pathogenicity test and in different *in vivo* experiments on botanicals, organic preparations, bio-control agents and fungicides evaluation. Seed of wheat germplasm (200 lines) for identification of

resistant lines to FHB was obtained from CSKHPKV, Rice and Wheat Research Center, Malan. These germplasm lines included released varieties from different agroclimatic zones, advanced breeding lines and few lines from national and international nurseries from CIMMYT and ICARDA (APPENDIX III).

3.1.9 Fungicides

Nine fungicides as enlisted below in the table were obtained from the Department of Plant Pathology and evaluated at different concentrations under *in vitro* experiment. The potential ones were further evaluated for the management of FHB in a pot experiment.

Sr no.	Chemical name	Trade name	Concentrations (ppm)
1	Mancozeb 75WP	Indofil	50, 100, 250, 500, 750, 1000, 1500 and 2000
2	Copper oxychloride 50 WP	Blitox	50, 100, 250, 500, 750, 1000, 1500 and 2000
3	Propiconazole 25 EC	Tilt	50, 100, 250, 500, 750, 1000, 1500 and 2000
4	Tebuconazole 250 EC	Folicur	50, 100, 250, 500, 750, 1000, 1500 and 2000
5	Difenaconazole 25 EC	Score	50, 100, 250, 500, 750, 1000, 1500 and 2000
6	Carbendazim 50 WP	Bavistin	50, 100, 250, 500, 750, 1000, 1500 and 2000
7	Carboxin 37.5% +thiram 37.5% WS	Vitavax power	50, 100, 250, 500, 750, 1000, 1500 and 2000
8	Trifloxystrobin 25% + tebuconazole 50% WG	Nativo	50, 100, 250, 500, 750, 1000, 1500 and 2000
9	Carbendazim 12% + mancozeb 63% WP	SAAF	50, 100, 250, 500, 750, 1000, 1500 and 2000

3.2 Methods

3.2.1 Survey and collection of diseased samples

Extensive surveys of major wheat growing areas of Himachal Pradesh were carried out during the period of March-April in 2019-20 and 2020-21, to record the status of the Fusarium head blight disease and to collect infected plant material for isolation of the pathogen(s). An area of one square meter each was marked at five spots along the diagonal of each field for recording the incidence/ severity of the disease. Incidence was recorded as the proportion of diseased spikes, while severity was recorded as the average proportion of diseased spikelets per spike (Paul et al. 2005).

The spikes showing the characteristics symptoms of Fusarium head blight were collected, placed in paper bags and brought to the laboratory for isolation purpose.

3.2.2 Isolation and identification of the pathogen

Wheat grains taken out from the infected earheads were used to isolate the pathogen(s). The isolation was done by Agar plate method using Potato Dextrose Agar (PDA) as the basal medium. The grains were surface sterilized with 1 per cent sodium hypochlorite solution for one minute and then washed thrice with sterilized distilled water under the laminar air flow. The grains were dried under the fold of sterilized blotting paper to remove the excessive moisture/ water and four grains were placed in each Petri plate containing sterilized and solidified PDA medium with the help of a sterilized forcep. The plates were incubated in the BOD incubator at $25\pm 1^{\circ}\text{C}$ and observed regularly for the formation of colonies with the characteristic growth of the pathogen(s). The pathogen(s) (*Fusarium* spp.) showing characteristic fungal characters in colonies were further sub-cultured on PDA plates and incubated in BOD incubator at $25\pm 1^{\circ}\text{C}$.

Fungal cultures were purified by using single hyphal tip method (Dhingra and Sinclair 1985). The mycelial bits were cut from the margins of actively growing colony from fresh culture and were transferred into Petri plates containing sterilized water agar (2%) medium with sterilized inoculation needle under aseptic conditions in

laminar air flow and incubated at $25\pm 1^{\circ}\text{C}$ in BOD incubator for 72 hours. The single well isolated hyphal tip growing in water agar medium was located under the microscope and marked with fine tip marker. The marked portion was cut with sterilized cork borer and transferred to PDA slant using inoculation needle and incubated at $25\pm 1^{\circ}\text{C}$ in a BOD incubator. The fungal colony obtained from single hypha represented the pure culture of the pathogen. The pure cultures were preserved at 4°C in refrigerator for further studies. Revival of pure cultures was done at regular time intervals.

The identity of *Fusarium* species as isolated above, was established on the basis of morphological and cultural parameters described by Booth (1971) and Leslie and Summerell (2006) given in Table 3.1. The microscopic examination of various fungal isolates in the present study was usually done by mounting mycelium in the water on a clean slide.

3.2.3 Pathogenicity test

Preparation of inoculum

Conidial suspensions of *Fusarium* species were prepared individually in sterile distilled water for artificial inoculation of earheads of wheat. For the preparation of conidial suspension, freshly sporulating fungal colonies of each isolate in Petri plates were washed with 20 mL of sterile distilled water, shaken gently and scrapped with sterilized spatula. The concentration of macroconidia in each suspension was adjusted to 5×10^5 spore/ mL with the help of hemocytometer. This concentration was used as standard inoculum for performing pathogenicity test.

Inoculation

The pathogenic behavior of the isolated *Fusarium* spp. was ascertained by inoculating healthy spikes of wheat variety HPW 89. The artificial inoculation of healthy spikes of wheat plants raised in plastic pots was done by spray inoculation method using homogenized spore suspension (5×10^5 spore/ mL) of each *Fusarium* spp. The inoculated spikes were immediately covered with thin wet (internal side) polythene bags for 72 hours and the pots were kept in a green house. Proper humidity was maintained in the polythene bag by frequently spraying water on the spikes till the development of typical disease symptoms.

Table 3.1 Parameters used for the identification of *Fusarium* species causing Fusarium head blight of wheat

Sr. no.	Pathogen species	Colony Characters	Macro Conidia	Micro conidia	Description of conidia	Size of conidia	References
1.	<i>Fusarium graminearum</i>	Dense mycelia which varies from white, pale pink to orange to yellow in colour	Present	Absent	Slender, thick walled, apical cell tapered, well developed foot cell, 5-6 septa	24.0–72.0 × 4.0–7.0 µm	Booth (1971)
2.	<i>Fusarium compactum</i>	White mycelia that may become pigmented (brown to red) with age	Present	Absent	Spindle shaped, thick walled with strong dorsi-ventral curvature, wide at the mid point, elongated and tapering apical cell, basal cell pronounced and distinct foot shape, 5 septa	25.0–35.0 × 3.0–5.0 µm	Leslie and Summerell (2006)
3.	<i>Fusarium avenaceum</i>	loosely fluffy to cottony aerial mycelia, which varies from white to light yellow to grayish rose	Present	Present	Slender, thin walled, straight to slightly curved macroconidia, apical cell long and tapering to a point may be bent, Basal cell usually notched may have foot shaped basal cell, 3-5 septa, fusoid microconidia 1-2 septa	Macroconida 45.5–65.5 × 3.5–4.5 µm microconidia 13.4-24.6 × 2.6–4.8 µm	Leslie and Summerell (2006)

The earheads were regularly observed for the appearance of symptoms.

Re-isolation

The causal organisms were re-isolated from the artificially inoculated spikes showing typical symptoms. The fungal growth obtained on PDA after re-isolation was compared with the original culture obtained from naturally infected spikes under field condition. The re-isolated pure fungal cultures were identified by comparing their morphological/ colony characters with the information available in the literature for fungal identification.

The most prevalent pathogen *Fusarium graminearum* was further used to conduct all the experiments.

3.2.4 Factors affecting pathogen growth and development

3.2.4.1 Effect of temperature

The effect of different temperature levels viz., 10, 15, 20, 25, 30 and 35°C on mycelial growth, sporulation and perithecia formation of the pathogen was studied under *in vitro* conditions. The mycelial discs of 5 mm diameter were cut with the help of a cork borer from the margin of actively growing colony of *Fusarium graminearum* and inoculated into 90 mm Petri plates containing 20 mL of solidified PDA medium and then incubated at different temperature regimes viz., 10, 15, 20, 25, 30 and 35°C in BOD incubators. Each treatment was replicated thrice in a Completely Randomized Design (CRD). After seven days of incubation, the colony diameter was measured with a scale and sporulation (conidia/ mL) assured with the help of a haemocytometer.

Perithecia formation in cultures was ascertained using the method of Klittich and Leslie (1988) with some modifications. The cultures were grown on PDA in 9 cm diameter Petri dishes at different temperatures mentioned above under 24 h lighting of a 15 W fluorescent bulb. After seven days of incubation, 1.0 mL of 2.5% Tween 60 (Sigma) was poured over the culture in each plate and spread across the surface with a sterile glass rod and then cultures were again incubated at respective temperatures until the formation of perithecia. The cultures were regularly observed for the formation of perithecia. Perithecia formation was recorded 12 days post treatment by visual estimate.

3.2.4.2 Effect of pH

The effect of five pH levels viz., 4, 5, 6, 7 and 8 was studied on the mycelial growth, sporulation and perithecia formation of the pathogen under *in vitro* conditions. Different pH levels of PDA medium were maintained by following the method described by Sharma et al. (2005). The pH levels of the medium were adjusted using HCl (0.1N) and NaOH (0.1N) before autoclaving. After autoclaving the medium with different pH levels were poured 20 mL each individually in Petri plates (90 mm) and allowed to solidify. The Petri plates were then inoculated centrally with mycelial discs (5 mm dia) cut from the actively growing culture of *Fusarium graminearum*. Each treatment was replicated thrice in a Completely Randomized Design (CRD). Inoculated Petri plates were incubated at a temperature of $25\pm1^{\circ}\text{C}$ in the BOD incubator. After seven days of incubation, the colony diameter was measured with a scale and sporulation (conidia/ mL) recorded with the help of a haemocytometer.

Perithecia formation in culture was determined by following the method of Klittich and Leslie (1988) with some modifications. The cultures maintained at different pH levels as mentioned above (4, 5, 6, 7 and 8) were incubated at room temperature under a 24 h lighting of 15 W fluorescent bulb. After seven days of incubation, 1.0 mL of 2.5 % Tween 60 (Sigma) was poured over the culture in each Petri plate and spread across the surface with a sterile glass rod and then cultures were again incubated at room temperature until the formation of perithecia. The cultures were regularly observed for the formation of perithecia and their final number was recorded 12 days post treatment by visual estimate.

3.2.5 Factors affecting disease development

3.2.5.1 Source of inoculum

To ascertain the role of crop residue, soil and seed as primary source of inoculum, soil samples, seed and stubbles of cereal crops (maize, wheat and rice) from the infected crop fields were collected at the end of the *kharif*, *rabi* and *kharif* season, respectively. Isolations were made from them to isolate different *Fusarium* spp. Isolation of *Fusarium* spp. from the seed of wheat, maize and rice was made as described under section 3.2.2.

Isolation of *Fusarium* spp. from crop residue of wheat, maize and rice

Small bits of size 2-3 mm having infected and healthy tissue were cut from the stubbles with the help of sharp sterilized blade. These bits were then disinfected with 1 per cent sodium hypochlorite solution for 20-30 seconds followed by three washings with distilled sterile water to remove the traces of chemical. The bits were dried under the fold of sterilized blotting paper to remove excessive moisture/ water and aseptically inoculated into Petri plates containing sterilized and solidified PDA medium with the help of sterilized forceps/ inoculation needle. The plates were incubated at $25\pm 1^{\circ}\text{C}$ in the BOD incubator and observed regularly for the appearance of colonies with the characteristic growth of *Fusarium* species. The colonies with characteristic growth of *Fusarium* spp. were observed under the stereoscopic microscope and mycelial growth from these sub-cultured on PDA plates. Fungal cultures were purified by using single hyphal tip method and pure cultures in PDA slants preserved at 4°C in a refrigerator for further studies.

Isolation of *Fusarium* spp. from soil samples of wheat, maize and rice

Isolation of *Fusarium* spp. from the soil samples was made by using serial dilution plate technique (Dhingra and Sinclair 2000). In this method, 10 g of soil was aseptically transferred into a 250 mL conical flask containing 100 mL of sterile distilled water and the contents were mixed thoroughly by shaking for five minutes. An aliquot of 10 mL with pipette was drawn from the mixture and transferred to 90 mL of sterile distilled water. The suspension was shaken thoroughly for one minute before its further dilution. Further dilutions of 10^{-4} to 10^{-6} were obtained in a similar way and used for the isolation of fungi. Sterilized PDA medium, supplemented with a pinch of streptomycin (before pouring) to avoid growth of bacterial colonies, 20 mL each was poured in a Petri plate and allowed to solidify. After the solidification of amended medium, 0.1 mL of suspension (from respective dilutions) were transferred aseptically into these plates and spread over the surface by swirling them gently. The plates were incubated at $25\pm 1^{\circ}\text{C}$ in the BOD incubator and observed regularly for the presence of colonies with characteristic growth of *Fusarium* species. The colonies with characteristic growth of *Fusarium* spp. were observed under the stereoscopic microscope and mycelial growth from such colonies was sub-cultured on PDA plates.

Fungal cultures were further purified by using single hyphal tip method and pure cultures in PDA slants were preserved at 4°C in a refrigerator for further studies.

Fusarium spp. isolated from seed, stubbles and soil of wheat were represented as W-1, W-2 and W-3, maize were represented as M-1, M-2 and M-3 and rice were represented as R-1, R-2 and R-3, respectively. The pathogenic behaviour of these *Fusarium* spp. was further evaluated by cross inoculation of wheat, maize and rice plants under pot conditions.

3.2.5.2 Effect of weather parameters on disease development

Effect of weather variables such as temperature, relative humidity and rainfall on the progression of FHB of wheat at three different dates i.e. 31st Oct, 15th Nov and 1st Dec was studied under field conditions. The field experiment was conducted at Rice and Wheat Research Center (RWRC), Malan by sowing artificially inoculated seed of wheat variety HPW 89 in randomized block design with four replications during *Rabi* season of 2019-20 and 2020-21. Seeds were inoculated by dipping in conidial suspension (5×10^5 spores/ mL) of *Fusarium graminearum* for 15 minutes and then shade dried overnight and sown on the next day. Inoculated seeds were sown in plots of 1.0×1.0 m² size with row to row spacing of 20 cm. The recommended agronomical practices were followed for conducting this experiment. The temporal progress of the disease in terms of disease severity (%) was recorded after every 10th day starting from the first appearance of the disease on ten randomly selected spikes. The data on agrometeorological factors viz., temperature, relative humidity and rainfall was taken from the RWRC, Malan. Disease severity (%) on spikes of crop of each sowing date was correlated with the meteorological factors (mean temperature, mean RH, total rainfall) to obtain simple, partial and multiple correlations, regression equations and coefficients of determination (R^2).

3.2.5.3 Incubation and Latent period

Incubation and latent period of *Fusarium graminearum* was studied by spray inoculating the earheads of a susceptible wheat variety (HPW 89) with the conidial suspension containing 5×10^5 spores/ mL. The inoculated spikes were immediately covered individually with a wet (internal side) thin polythene bag for 72 hours and the pots were kept in the green house. Proper humidity was maintained in the bags by

frequent spraying of water on the spikes till the development of typical disease symptoms. The plants were regularly observed for the appearance of first symptoms and sporulation of the fungus on the lesion.

3.2.5.4 Host range of the pathogen

The pathogenic behaviour of *Fusarium graminearum* was studied by inoculating plants of five cereal crops viz., maize (*Zea mays*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), rye (*Secale cereale*) and oats (*Avena sativa*). Surface sterilized seeds (with 1% sodium hypochlorite) of these crops were sown in pots containing sterilized soil and three replications of each were maintained and kept in the greenhouse. The plants were spray inoculated with the help of an atomizer at inflorescence stage (except maize) with conidial suspension containing 5×10^5 spores/mL of *F. graminearum*. Whereas, in maize one month old plants were spray inoculated with conidial suspension (5×10^5 spores/mL). Control plants of these crops were sprayed with sterile distilled water. After inoculation, plants were covered with polythene bags for 48 hours to provide optimum conditions for the infection by the pathogen. The plants were observed regularly for the appearance of any symptoms.

3.2.6 Identification of resistance sources

Wheat germplasm comprising of 200 different varieties and lines was obtained from Rice and Wheat Research Center (RWRC), Malan and evaluated for resistance to FHB disease under artificially inoculated conditions at its research farm during Rabi 2019-20 and 2020-21. Seeds of individual variety/ line were inoculated by dipping in conidial suspension (5×10^5 spores/mL) of *Fusarium graminearum* for 15 minutes and then shade dried overnight and sown on the next day in 1.0 m row. The recommended agronomic practices for wheat crop were followed for conducting this experiment.

The germplasm was spray inoculated with conidial suspension containing 5×10^5 spores/mL at anthesis stage during the evening hours. Ordinary water was sprayed over the earheads of the varieties/ lines with the help of a sprayer in the evening for the next 5 days to maintain the moisture. The data on terminal disease severity (%) were recorded post 30 days of inoculation.

The evaluated varieties and lines were categorized for resistance to FHB according to 0-5 scale employed by Ireta and Gilchrist (1994).

Rating	% spikelet infection	Category
0	0	Free
1	1-5	Resistant (R)
2	5-25	Moderately Resistant (MR)
3	25-50	Moderately Susceptible (MS)
4	50-75	Susceptible (S)
5	>75	Very Susceptible (VS)

3.2.7 Management of the disease

3.2.7.1 Effect of different sowing dates

The effect of different sowing dates i.e. early (31st October), timely (15th November) and late (1st December) on disease development was evaluated in a pot experiment by using seed of susceptible wheat variety (HPW 89). Seeds were inoculated by dipping in conidial suspension (5×10^5 spores/ mL) of *Fusarium graminearum* for 15 minutes and then shade dried overnight and sown on the next day in pots containing sterilized soil. Five seeds were planted in each pot and four replications of each treatment were maintained under green house. The data on terminal disease incidence (%) and severity (%) were recorded.

3.2.7.2 *In vitro* evaluation of organic and natural preparations/ amendments

Nine organic and natural preparations/ amendments viz., Vermiwash, Beejamrit, Jeevamrit, Brahmastra, Tamarlassi, Eupatorium plant extract, Biosol, Neem and Mustard cakes were evaluated against test pathogen (*F. graminearum*) through poisoned food technique (Nene and Thapliyal 2000) under *in vitro* conditions.

First of all 100 g neem and mustard cakes were dissolved in 100 mL of distilled sterilized water and then aqueous extract was obtained by filtration. The

aqueous extract was collected by filtration through Whatman No.1 filter paper and the filtrate so obtained was used as 100 per cent stock solution. The stock solutions of these preparations were serially diluted with distilled water to obtain the desired concentrations of 10, 20, 30, 40 and 50 per cent. These concentrations (50 mL each) were then mixed individually with equivalent amount of double strength sterilized PDA medium to get test concentrations (10, 20, 30, 40 and 50 %) and poured aseptically in Petri plates. The double strength medium mixed with an equal quantity of sterilized distilled water served as control. With the help of a sterilized cork borer, 5 mm diameter mycelial bits were cut from the margin of an actively growing colony and each placed in the centre of media plates. Each treatment was replicated thrice and incubated at a temperature of $25\pm 1^{\circ}\text{C}$ in the BOD incubator. Regular observations were made for the growth of the pathogen and finally colony diameters were measured when the control plates were completely covered by the fungal growth and per cent mycelial inhibition calculated by using the formula given by Vincent (1947):

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,

C= Mycelial growth of fungus in control (mm)

T= Mycelial growth of fungus in treatment (mm)

3.2.7.3 *In vitro* evaluation of botanicals

The aqueous and alcoholic extracts of eleven botanicals viz.; *Azadirachta indica*, *Melia azedarach*, *Eucalyptus tereticornis*, *Eupatorium adenophorum*, *Ranunculus muricatus*, *Euphorbia royleana*, *Vitex negundo*, *Lantana camara*, *Polygonum hydropiper*, *Withania somnifera* and *Acorus calamus* were evaluated against test pathogen using poisoned food technique.

Leaves of first ten botanicals and rhizomes of *A. calamus* were collected from adjoining areas, shade dried and ground with a blender to obtain fine powder of each. The powders so obtained were stored in clean plastic bags at room temperature with proper labelling. The aqueous and alcoholic extracts of above botanicals were prepared as follow:

Aqueous extract: 100 g of each powdered botanical was soaked overnight in 200 mL of sterilized distilled water (1:2 w/v) in 500 mL conical flask. The extract obtained after 4 hours was filtered through a double layer of muslin cloth and twice through Whatman No. 1 filter paper to get the clear filtrate. This was considered as the standard aqueous extract of a botanical. The antifungal properties of these extracts was studied by Poisoned food technique.

Alcoholic extract: 100 g of each powdered sample of botanical was suspended in 200 mL of methanol in a 500 mL conical flask, plugged with a cotton plug and kept on a rotary shaker at 190-220 rpm for 24 hrs. The supernatant of each botanical extract was collected and kept for evaporation in open to make the final volume one fourth of the original one and then, stored in an air tight bottle at 4 °C in refrigerator.

The stock solutions (aqueous/ alcoholic) of botanicals were serially diluted with distilled water to obtain the desired concentrations of 10, 20, 30, 40 and 50 per cent. These concentrations (50 mL each) were then mixed individually with equivalent amount of double strength sterilized PDA medium to get test concentrations (10, 20, 30, 40 and 50 %) and poured aseptically in Petri plates. The double strength medium mixed with an equal quantity of sterilized distilled water served as control. Mycelial bits (5 mm dia) were cut with the help of a sterilized cork borer from the margin of an actively growing colony and each placed in the centre of media plates. Each treatment was replicated thrice in a completely randomized design and incubated at a temperature of $25 \pm 1^\circ\text{C}$ in the BOD incubator. The Petri plates were observed regularly for the growth of the pathogen and finally colony diameters were measured when the control plates were completely covered by the fungal growth and per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

3.2.7.4 *In vitro* evaluation of biocontrol agents

Isolation of *Trichoderma* spp.

The fungal bioagents were isolated from the soil samples taken from the wheat rhizosphere by using serial dilution plate technique (Dhingra and Sinclair 2000) as described in section 3.2.6.1. The colonies with characteristic growth of *Trichoderma* spp. were observed and marked under the stereoscopic microscope and then the

mycelial growth from such colonies was sub-cultured on agar plates. The fungal bioagents (*Trichoderma* spp.) were further purified by using single hyphal tip method and pure cultures in PDA slants were preserved at 4°C in a refrigerator for further studies.

Six isolates of *Trichoderma* spp. (T-1, T-2, T-3, T-4, T-5 and T-6) along with four standard *Trichoderma* spp. procured from the department viz., *T. viride* (Tv), *T. harzianum* (TH-5), *T. koningii* (DMA-8) and *T. koningii* (JMA-11) and one bacterial bioagent (*Pseudomonas fluorescens*) were evaluated against the test fungus (*Fusarium graminearum*) by dual culture technique (Dennis and Webster 1971) and bacterium by streak plate method (Utkhede and Rahe 1983) under *in vitro* conditions. In dual culture seven days old culture discs (5 mm dia) of each fungal antagonist and the pathogen were taken from the margin of their actively growing culture with the help of sterilized cork borer and transferred aseptically to solidified PDA contained in Petri plates (90 mm) by placing them on the same dish opposite to each other (7 cm apart from each other) at an equal distance of 1 cm from the periphery of the plate. The Petri plate containing only culture disc of the pathogen served as control. While in case of bacterial antagonist, its inoculum was streaked at one side (one cm away from the edge) and a mycelial bit of the test fungus (7 days old culture) placed at the opposite side, one cm away from the edge of the Petri plate. Each treatment was replicated thrice in CRD design. The inoculated plates were incubated at 25±1°C in a BOD incubator. The radial growth of the test pathogen and inhibition zone was measured after 7 days of inoculation with the help of a scale and per cent inhibition was calculated by using formula given by Vincent (1947).

3.2.7.5 *In vitro* evaluation of fungicides

Nine fungicides viz., trifloxystrobin 25% + tebuconazole 50% (Nativo 75WG), carbendazim 12% + mancozeb 63% WP (SAAF), difenaconazole 25 EC (Score), carbendazim 50 WP (Bavistin), Propiconazole 25 EC (Tilt), tebuconazole 250 EC (Folicur), mancozeb 75WP (Indofil), copper oxychloride 50 WP (Blitox) and carboxin 37.5% +thiram 37.5% WS (Vitavax power) were evaluated at 50, 100, 250, 500, 750, 1000, 1500 and 2000 ppm concentrations for their efficacy against test pathogen by poisoned food technique (Nene and Thapliyal 2000).

Double strength PDA medium was prepared in distilled water and sterilized in autoclave at 1.05 kg/ cm³ pressure (121±1°C) for 20 minutes. Simultaneously, double strength concentrations of fungicides were also prepared in sterilized distilled water and mixed individually with the double strength medium so that the desired concentrations are obtained after mixing of both. Then the contents were poured in Petri plates and allowed to solidify. Mycelial bits (5 mm dia) were cut with the help of a sterilized cork borer from the margin of an actively growing colony and each placed in the centre of media plates. A control treatment was also maintained in which only plain sterilized distilled water was added to double strength medium. Each treatment was replicated thrice in CRD design. The inoculated plates were incubated at 25±1°C in a BOD incubator.

Regular observations were made for the growth of *F. graminearum* and finally colony diameters were measured with a scale when the control plates were completely covered by the pathogen and per cent inhibition of mycelial growth was calculated by using formula given by Vincent (1947).

3.2.7.6 Evaluation of potential organic preparations, botanicals, biocontrol agents and fungicides *in vivo*

Based on the *in vitro* performance, three most effective treatments each of organic preparations (Beejamrit, Jeevamrit and Vermiwash), aqueous extracts of botanicals (*Acorus calamus*, *Withania somnifera* and *Vitex negundo*), biocontrol agents (*Trichoderma harzianum* (TH-5), *T. koningii* (DMA-8) and *T. viride* (Tv)), fungicides (carbendazim 50 WP, trifloxystrobin 25% + tebuconazole 50% WG and tebuconazole 250 EC) were further evaluated as seed treatment and foliar sprays against FHB in a pot experiment by using infected seed. Seeds were inoculated by dipping in conidial suspension (5×10⁵ spores/ mL) of *Fusarium graminearum* for 15 minutes and then shade dried overnight. In case of organic preparations and botanicals, seed treatment was done by mixing @ 10 mL/ kg seed. In biocontrol agents talc formulations were used to treat the seed @ 10 g/ kg. The seeds were placed in a sterilized container and added desired quantity of talc formulation of bioagent and agitated until the seeds were uniformly primed. In case of fungicides,

seeds were treated with individual fungicide @ 2 g or mL/ kg seed. The experiment was conducted in plastic pots with completely randomized design (CRD).

Five treated seeds from each treatment (organic preparations, botanicals, biocontrol agents and fungicides) were planted in each pot and each treatment was replicated four times. At the appearance of first symptoms of FHB, first foliar application of three organic preparations (10 mL/ L water), three botanicals (10 mL/ L water), three biocontrol agents (10 mL/ L water) and three fungicides (1 g or mL/ L water) was given. The second spray was given after 10 days of the first application. Pots without any treatment served as check. Plants in pots were watered regularly to maintain proper moisture. The data on disease incidence (%) and severity (%) were recorded and per cent reduction in disease incidence and severity over the check were calculated.

3.2.7.7 Integration of IDM components under field conditions

The best treatments from different management components (botanicals, organic preparations, biocontrol agents and fungicides) were integrated to evaluate their combined effect on the control of FHB in a field trial.

Seeds of wheat variety (HPW 89) were inoculated by dipping in conidial suspension (5×10^5 spores/ mL) of *Fusarium graminearum* for 15 minutes and then shade dried overnight. On the next day the seeds were treated with individual component as given below and sown in the plots of size $1.5 \times 1.5 \text{ m}^2$. The trial with the following treatments each replicated thrice was laid out in RBD design at RWRC, Malan during 2020-2021.

Detail of treatment combinations

Treatments	
T1	Seed treatment with carbendazim 50 WP @ 2 g/ kg seed + two foliar sprays of carbendazim 50 WP @ 1 g/ L water
T2	Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/ kg seed + two foliar sprays of <i>Acorus calamus</i> @ 10 mL/ L water
T3	Seed treatment with Beejamrit @ 10 mL/ kg seed + two foliar sprays of Jeevamrit @ 10 mL/ L water
T4	Seed treatment with carbendazim 50 WP @ 2 g/ kg seed + foliar spray of

	Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water
T5	Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/ kg seed + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water
T6	Seed treatment with Beejamrit @ 10 mL/ kg seed + foliar spray of <i>Acorus calamus</i> @ 10 mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water
T7	Seed treatment with carbendazim 50 WP @ 2 g/ kg seed + foliar spray of Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water
T8	Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/ kg seed+ foliar spray of Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water
T9	Seed treatment with Beejamrit @ 10 mL/ kg seed + foliar spray of Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @1 g/ L water
T10	Check

Foliar application of each management component (botanical, organic preparation and fungicide) were given individually at 10 days interval. The first spray was given at the first appearance of the disease. Plants sprayed with water served as control. The data on disease incidence (%) and severity (%) were recorded before each spray and 10 days after the last spray.

Area under Disease Progress Curve

The AUDPC values were calculated using the formulae given by Wilcoxson et al. (1975).

$$AUDPC = \sum_{i=0}^k \left(\frac{Y_i + Y_{i-1}}{2} \right) \times d$$

Where AUDPC = Area under Disease Progress Curve

Y_i = Disease severity at the end of time i

k = Number of successive evaluation of blight severity

d = d is the interval between i and $i-1$ evaluation of the disease

Apparent rate of infection (r):

Rate of infection was calculated by adopting the formula given by Vanderplank (1963).

$$r = \frac{2.3}{t_2 - t_1} \log \frac{X_2}{X_1}$$

Where, r = rate of disease progress or infection rate

$t_2 - t_1$ = time interval

X_1 = Disease at time t_1

X_2 = Disease at time t_2

Statistical Analysis

The data recorded were subjected to statistical analysis where ever required. The differences exhibited by the treatments in various experiments were tested for their significance by employing Completely Randomized Design (CRD) and/ or Randomized Block Design (RBD) as per the details given by Gomez and Gomez (1984). All the data were analyzed by using CPCS-1, MS, SPSS and OPSTAT.

4. RESULTS AND DISCUSSION

The results obtained on various aspects of the present investigation entitled “**Epidemiology and management of Fusarium head blight of wheat**” conducted at the Department of Plant Pathology, COA, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur and Rice and Wheat Research Centre (RWRC), Malan during 2019-21 are presented and discussed under the following Sub-headings:

4.1 Status and distribution of the disease

The data on disease incidence and severity of Fusarium head blight (FHB) of wheat at different locations was recorded by conducting survey of main wheat-growing districts viz., Kangra, Una, Sirmour, Hamirpur, Mandi and Bilaspur of Himachal Pradesh during *Rabi* season of two consecutive years i.e. 2019-20 and 2020-21 and presented in Table 4.1 and Figure 4.1. The data reveal that the disease was prevalent at all the locations surveyed, however, its incidence and severity varied from location to location. The maximum average disease incidence and severity were observed in district Una (7.74 & 25.29 %) followed by Kangra (5.94 & 24.19 %), Bilaspur (5.89 & 20.71 %), Sirmour (5.71 & 19.38 %) and Hamirpur (4.53 & 17.84 %), respectively. However, the least average disease incidence and severity were recorded in district Mandi (3.25 & 15.08 %), respectively. Among all the surveyed locations, the mean disease incidence and severity were found to be maximum at Duhki (12.22 & 33.85 %) of district Una followed by Malan (10.77 & 31.34 %) of district Kangra, Gehrwin (9.35 & 27.78 %) of district Bilaspur, Dhaulakuan (6.72 & 22.71 %) of district Sirmour, Khidki (6.46 & 20.95 %) of district Hamirpur and minimum at Mehar (2.31 & 13.37 %) of district Mandi, respectively. It is also found from the data that disease incidence and severity were higher at each location during 2020-21 than 2019-20. The varying incidence and severity of the disease at different locations and districts of the state may be attributed to the diversity in agroclimatic conditions and varieties used by the farmers. Andrews and Pitt (1986) reported that the occurrence of *Fusarium* species depended upon the agroclimatic conditions prevailing in different areas. The incidence of FHB on variety HPW89 was recorded as high as 17 per cent in Himachal Pradesh (Anonymous 1998).

Table 4.1 Incidence and severity of Fusarium head blight at different locations of Himachal Pradesh

District	Location	Disease incidence (%)			Disease severity (%)		
		2019-20	2020-21	Mean	2019-20	2020-21	Mean
Una	Akrot	4.17	5.24	4.71	17.30	22.40	19.85
	Bangana	5.34	7.34	6.34	23.58	25.71	24.65
	Duhki	11.36	13.07	12.22	32.92	34.77	33.85
	Chak	7.29	8.14	7.72	18.57	27.06	22.82
	Mean	7.04	8.45	7.74	23.09	27.49	25.29
Sirmaur	Dhaulakuan	6.29	7.14	6.72	19.51	25.90	22.71
	Kolar	4.28	5.12	4.70	13.87	18.23	16.05
	Mean	5.29	6.13	5.71	16.69	22.07	19.38
Kangra	Thural	3.21	4.79	4.00	19.40	21.63	20.54
	Malan	10.48	11.06	10.77	30.63	32.04	31.34
	Kangra	4.67	5.63	5.15	21.09	26.01	23.55
	Sai	3.79	3.94	3.86	17.81	24.92	21.37
	Mean	5.53	6.36	5.94	22.23	26.15	24.19
Bilaspur	Ghumarwin	3.45	3.72	3.62	10.28	15.50	12.89
	Berthin	4.38	5.03	4.71	17.60	25.33	21.47
	Gehrwin	8.59	10.10	9.35	26.65	28.90	27.78
	Mean	5.49	6.28	5.89	18.18	23.24	20.71
Mandi	Mehar	2.11	2.50	2.31	11.32	15.42	13.37
	Pakhari	3.65	4.72	4.19	14.50	19.06	16.78
	Mean	2.88	3.61	3.25	12.91	17.24	15.08
Hamirpur	Khidki	6.12	6.79	6.46	15.77	26.12	20.95
	Kaloor	2.15	3.06	2.61	12.65	16.80	14.73
	Mean	4.14	4.93	4.53	14.21	21.46	17.84
Overall mean		5.29	6.22	-	18.73	23.63	-

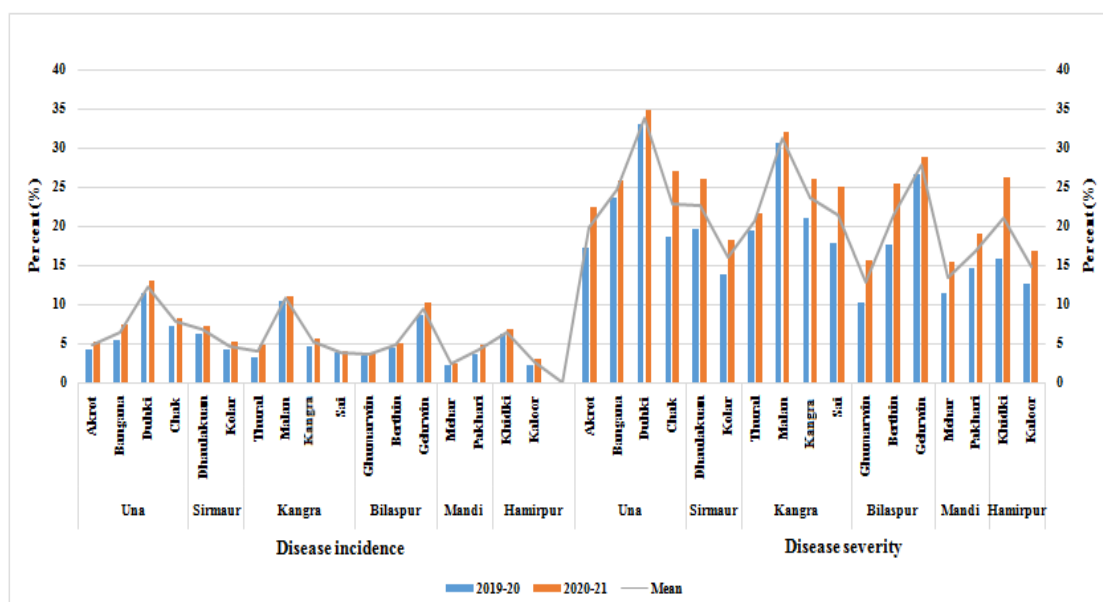


Figure 4.1. Disease incidence and severity of *Fusarium* head blight of wheat at different locations of Himachal Pradesh (2019-20 & 2020-21)

4.2 Isolation and identification of *Fusarium* species causing FHB of wheat

The fungi isolated from the diseased earheads of wheat were maintained as pure cultures on PDA slants. These were identified on the basis of their morphocultural characteristics viz., colony growth, colour, shape and size of spores and comparing/ confirming with standard descriptions/ keys (Table 4.2).

Table 4.2 Identification of *Fusarium* species associated with *Fusarium* head blight based on morphocultural characteristics

Sr. no.	Colony character	Macro conidia	Micro conidia	Conidial shape & size			Species identified
				Shape	Length* (µm)	Width *(µm)	
1.	Vary from white, yellow to pale pink coloured mycelium	Present	Absent	Canoe shaped with tapered apical cell, foot shaped basal cell, 5-6 septate	45.0-68.0	4.1-4.3	<i>Fusarium graminearum</i>
2.	Whitish woolly aerial mycelium	Present	Absent	Spindle shaped, thick walled, wide at mid point, tapering apical cell, pronounced foot cell, 5 septate	26.0-35.0	3.6-4.1	<i>Fusarium compactum</i>
3.	Fluffy aerial, yellow coloured mycelium	Present	Present	Slender, curved, apical cell pointed, basal cell notched, macroconidia, 3-5 septate, fusoid microconidia, 1-2 septate	Macroconidia 46.0-54.0 Microconidia 15.0-23.0	Macroconidia 3.7-4.1 Microconidia 2.8-3.9	<i>Fusarium avenaceum</i>

*average of 30 conidia

Isolate-1: *Fusarium graminearum*

Isolate-1 produced white, yellow to pale pink coloured aerial floccose mycelium, often becoming vinaceous with brown tinge in the later stages and beared only macroconidia (Plate 4.1a). Microscopically, the hyphae were hyaline, filamentous, septate and branched. The macroconidia were canoe shaped with tapered apical cell, basal cell foot shaped, 5-6 septate and measured $45.0-68.0 \times 4.1-4.3 \mu\text{m}$ in size (Plate 4.1b). These characteristics matched with those of *Fusarium graminearum* and thus isolate confirmed to be the same.

Isolate-2: *Fusarium compactum*

Isolate-2 produced whitish aerial mycelium with woolly appearance and beared only macroconidia (Plate 4.1c). Hyphae were hyaline, septate and branched. The macroconidia were spindle-shaped, thick walled, wide at mid point, tapering apical cell, pronounced foot cell, 5 septate and measured $26.0-35.0 \times 3.6-4.1 \mu\text{m}$ in size (Plate 4.1d). These characteristics resembled with those of *Fusarium compactum* hence, identified as the same.

Isolate-3: *Fusarium avenaceum*

Isolate-3 produced yellow coloured dense fluffy aerial mycelium (Plate 4.1e). Colony growth rate was rapid. The macroconidia were slender, 3-5 septate, $46.0-54.0 \mu\text{m}$ long and $3.7-4.1 \mu\text{m}$ wide (Plate 4.1f). Whereas, microconidia though formed rarely were fusoid with 1-2 septa and measured $15.0-23.0 \times 2.8-3.9 \mu\text{m}$ in size. The characters of the pathogen/ isolate observed were similar to *Fusarium avenaceum* thus, identified to be the same fungus.

Thus, three isolated fungi were identified as *F. graminearum*, *F. compactum* and *F. avenaceum* as their fungal characteristics and the morphology of the conidia resembled with the descriptions of Booth (1971) and Leslie and Summerell (2006).

Pathogenicity test

In pathogenicity tests, wheat spikes inoculated individually with freshly sporulating cultures of *F. graminearum*, *F. compactum* and *F. avenaceum* produced typical symptoms of the disease thus confirming the pathogenic behavior of the test pathogens. The inoculated spikes initially showed water soaked lesions at the base or middle of the glumes followed by salmon pink fungal growth along the edges of glumes. The inoculated spikes gave bleached appearance (Plate 4.2b). The infected grains were shriveled with floury discoloured interior (Plate 4.3). The latent and incubation periods of *Fusarium* species ranged between 7-8 and 9-11 days, respectively.

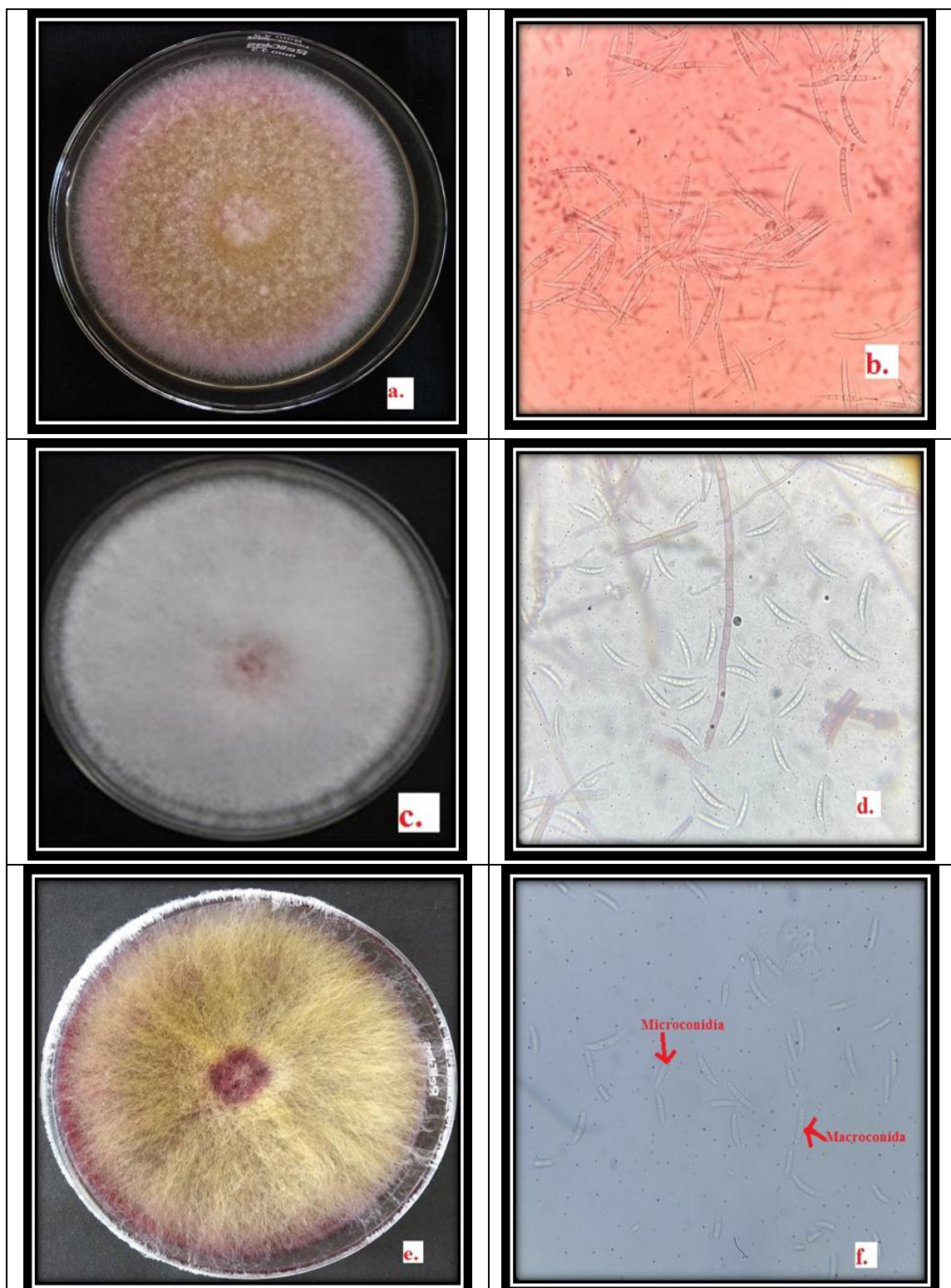


Plate 4.1: Morphocultural characteristics of *Fusarium graminearum* (a. mycelium and b. macroconidia), *F. compactum* (c. mycelium and d. macroconidia) and *F. avenaceum* (e. mycelium and f. macroconidia and microconidia)



Plate 4.2: Symptoms of Fusarium head blight on naturally infected spikes under field conditions (a) and artificially inoculated wheat spikes under greenhouse conditions (b)



Plate 4.3: Effect of *Fusarium graminearum* on wheat seed morphology

Prevalence of *Fusarium* species

It is evident from Table 4.3 that *F. graminearum* was the most prevalent species in Kangra, Una, Sirmour, Hamirpur, Mandi and Bilaspur districts of Himachal Pradesh. However, *F. compactum* and *F. avenaceum* were prevalent only in Kangra and Una districts, respectively. Hence, all the experiments were conducted with *F. graminearum*. Panwar et al. (2016) studied the distribution dynamics of *Fusarium* species causing FHB in wheat at different geographical locations of India and found that *F. graminearum* was predominant at all the sites surveyed.

Table 4.3 Prevalence of *Fusarium* species associated with Fusarium head blight in Himachal Pradesh

District	Prevalent <i>Fusarium</i> species		
	<i>Fusarium graminearum</i>	<i>Fusarium compactum</i>	<i>Fusarium avenaceum</i>
Hamirpur	+	-	-
Bilaspur	+	-	-
Mandi	+	-	-
Kangra	+	+	-
Sirmour	+	-	-
Una	+	-	+

'+' - Present

'-' - Absent

4.3 Factors affecting pathogen growth and development

4.3.1 Effect of temperature

Temperature plays a significant role in the growth and development of different plant pathogens. The data presented in Table 4.4 and Plate 4.4 reveal that *F. graminearum* grew at a wide range of temperature from 10 to 35°C with 25°C being the optimum temperature for mycelial growth of the pathogen. The maximum mycelial growth (90 mm) of the pathogen was recorded at 25°C followed by 30°C (87.60 mm). However, at 35, 20 and 15°C the pathogen showed only 48.00, 45.67 and 35.67 mm mycelial growth, respectively. The minimum mycelial growth (31.67 mm)

of the pathogen was recorded at 10°C. The mycelial growth decreased with the increase in temperature from 25 to 35°C and its decrease from 25 to 10°C.

It is also indicated by the data that temperature had a great effect on the sporulation (conidia/ mL) and perithecia formation. The highest sporulation (5.5×10^6 conidia/ mL) and high perithecia formation was observed at 25°C followed by 30°C with sporulation of 5.2×10^6 conidia per mL and less perithecia formation. However, lesser sporulation of 2.4×10^6 , 2.3×10^6 and 1.8×10^6 conidia per mL with moderate, nil and less perithecia formation were observed at 20, 35 and 15°C, respectively. The least sporulation (1.1×10^6 conidia/ mL) and very less perithecia formation were observed at 10°C.

Table 4.4 Effect of temperature on the growth and sporulation of *Fusarium graminearum*

Temperature (°C)	Mycelial growth* (mm)	Sporulation (Conidia/ mL)	Perithecia formation
10	31.67 (34.23)	1.1×10^6	Very less
15	35.67 (36.65)	1.8×10^6	Less
20	45.67 (42.50)	2.4×10^6	Moderate
25	90.00 (71.53)	5.5×10^6	High
30	87.60 (69.41)	5.2×10^6	Less
35	48.00 (43.84)	2.3×10^6	Nil
CD (p=0.05)	1.05	-	-

* Figures within parentheses are arc sign transformed values

The present findings are in accordance with the earlier observations of Tschanz et al. (1976) and Dufault et al. (2006) who also found that the optimum temperature for perithecia formation ranged from 15.0 to 28.5°C and 12.0 to 28.0°C, respectively. Rossi et al. (2002) revealed that the optimum temperature for macroconidia production was 32°C in *F. graminearum*. Brennan et al. (2003) and Hudec and Machova (2010) found 25°C to be the optimum temperature for the growth of *F.*

graminearum. Manstretta and Rossi (2016) observed that the perithecia were produced between a temperature range of 5-30°C with optimum of 21.7°C, whereas, no perithecia were formed at 35 to 40°C, and just a handful produced at 5°C.

4.3.2 Effect of pH

The pH of the medium is also responsible for affecting the growth and sporulation of plant pathogens. Perusal of the data (Table 4.5 and Plate 4.5) reveal that *F. graminearum* could grow at a wide range of pH from 4.0 to 8.0 with 5.0 being the optimum for mycelial growth of the pathogen. The maximum mycelial growth (86.00 mm) of the pathogen was recorded at pH 5.0 followed by 6.0 (81.33 mm). However, at pH 7.0 and 4.0 the pathogen showed only 74.00 and 46.67 mm mycelial growth, respectively. While, the minimum mycelial growth (30.33 mm) was recorded at pH 8.0.

It is also evident from the data that pH had a great effect on the sporulation (conidia/ mL) and perithecia formation of the pathogen. The highest sporulation of 5.7×10^6 conidia per mL and high perithecia formation were observed at pH 5.0 followed by 6.0 with sporulation of 5.3×10^6 conidia per mL and high perithecia formation. However, lesser sporulation of 3.6×10^6 and 2.4×10^6 conidia per mL with moderate and less perithecia formation were recorded at pH 7.0 and 8.0, respectively. The least sporulation (1.9×10^6 conidia/ mL) and very less perithecia formation was recorded at pH 4.0.

Table 4.5 Effect of pH on the growth and sporulation of *Fusarium graminearum*

pH level	Mycelial growth* (mm)	Sporulation (Conidia/ mL)	Perithecia formation
4	46.67 (43.07)	1.9×10^6	Very less
5	86.00 (68.00)	5.7×10^6	High
6	81.33 (64.38)	5.3×10^6	High
7	74.00 (59.32)	3.6×10^6	Moderate
8	30.33 (33.40)	2.4×10^6	Less
CD (p=0.05)	1.65	-	-

* Figures within parentheses are arc sign transformed values

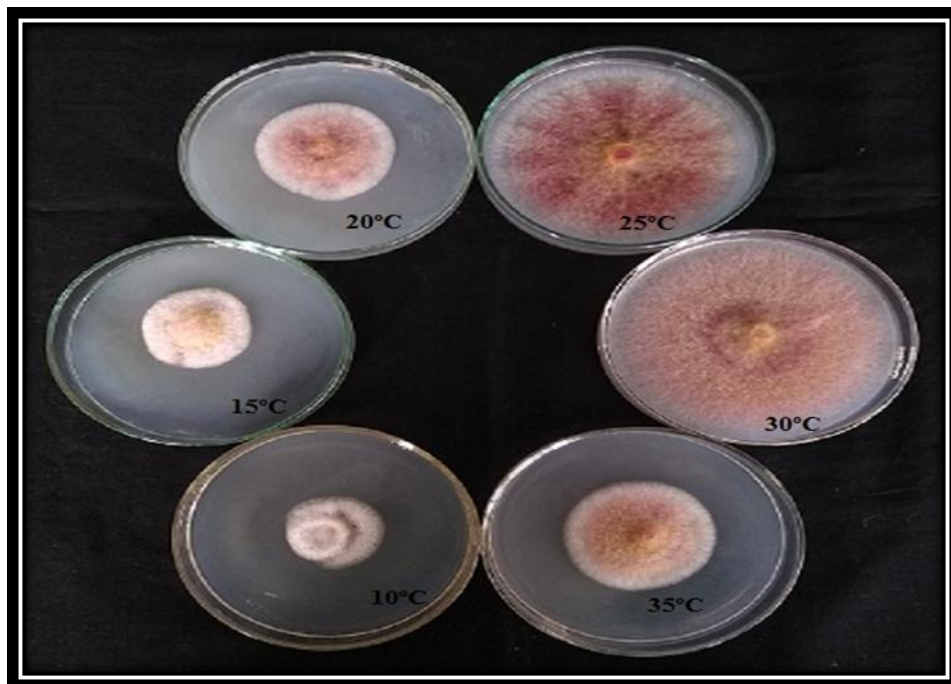


Plate 4.4: Effect of temperature on mycelial growth of *Fusarium graminearum*

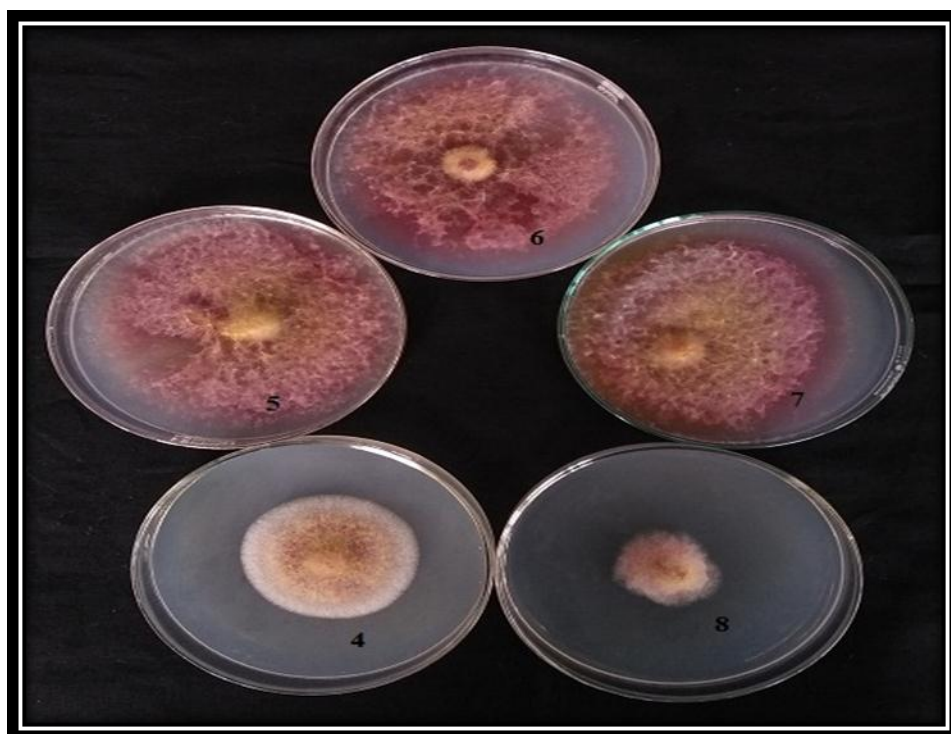


Plate 4.5: Effect of pH on mycelial growth of *Fusarium graminearum*

Several *Fusarium* species have been observed to grow and sporulate at a pH range of 5.0 to 6.0 (Cochrane 1958). Agarwal and Sarbhoy (1978) found that acidic pH favoured the growth of *Fusarium* species. Sharma et al. (2005) observed that the pH of 5.5 resulted in the highest mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *lini*. Similarly, Pal et al. (2019) noted the maximum mycelial growth (86.33 mm) and the highest sporulation (8.2×10^6 conidia/ mL) of *Fusarium oxysporum* f. sp. *lini* at pH 5.5.

4.4 Factors affecting disease development

4.4.1 Source of inoculum

The primary source of inoculum plays a major role in the initiation and development of plant diseases. It is apparent from Table 4.6 that isolates W-1, W-2, M-1, M-2, R-1 and R-2 showed pathogenic behaviour on all the three cereal crops viz., wheat, maize and rice. While, isolates W-3, M-3 and R-3 were found non-pathogenic on any of the crops.

Table 4.6 Pathogenic behaviour of *Fusarium* species isolated from different sources of inoculum

Isolate	Pathogenic behaviour on the crop		
	Wheat	Maize	Rice
W-1	+	+	+
W-2	+	+	+
W-3	-	-	-
M-1	+	+	+
M-2	+	+	+
M-3	-	-	-
R-1	+	+	+
R-2	+	+	+
R-3	-	-	-

W- Wheat, M-Maize, R-Rice

1- Seed, 2- Crop residue, 3- Soil

On wheat earheads, symptoms of FHB appeared initially as tan brown lesions at the base and middle of the glumes and then pink salmon spore masses were produced on infected spikelets. Infected spikelets exhibited premature bleaching of one or more spikelets located in the top, middle, or bottom or the entire head. Infected

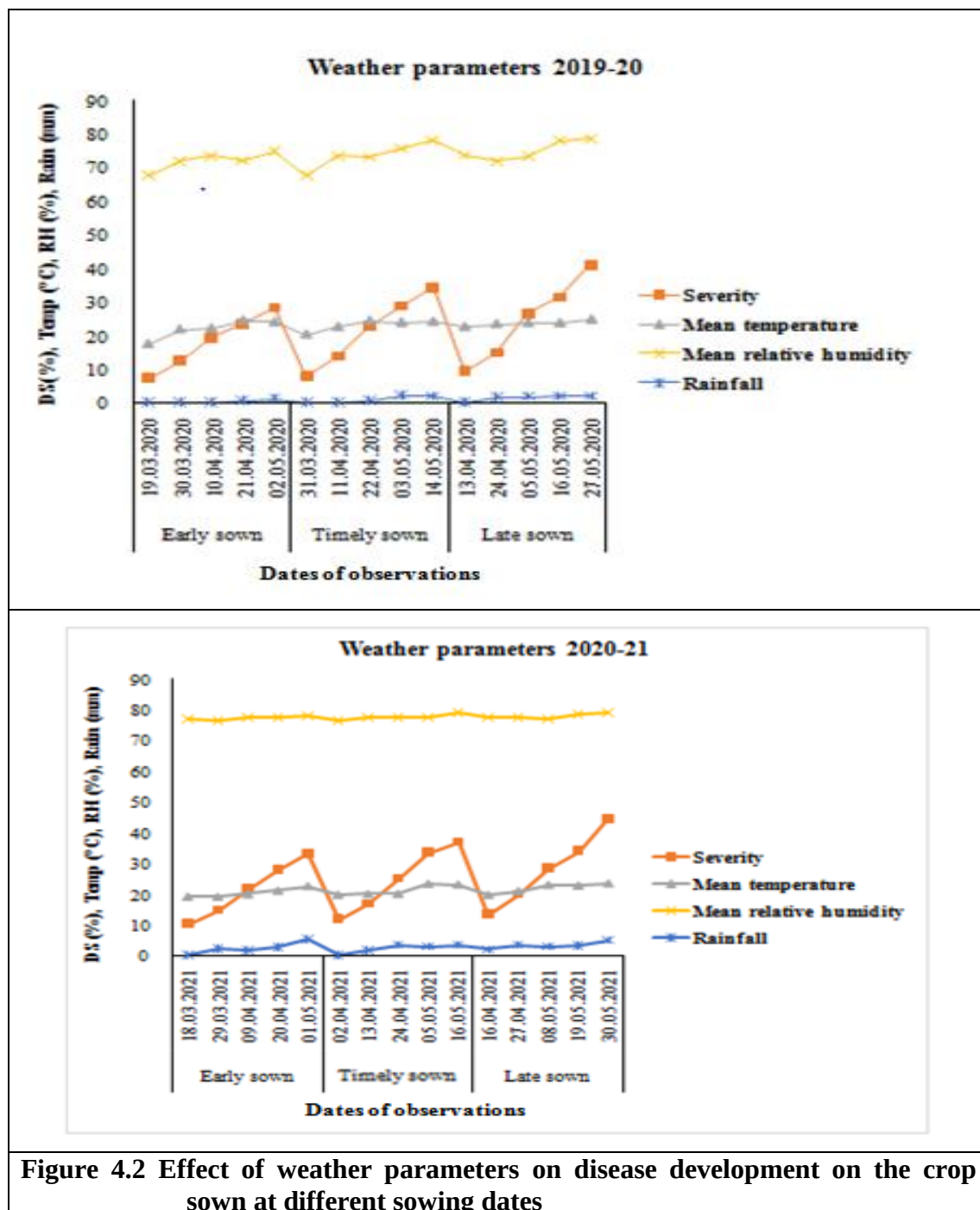
kernels were light in weight, chalky and shriveled. The blotching caused by this pathogen appeared on leaves at about late jointing to early booting. Young lesions occurred as oval to elliptical, grayish green mottled areas usually located at leaf bending. Whereas, in rice, initially small light brown spots were formed on glumes which later evolved into irregular brown lesions. A complete dark purplish staining or browning of the glumes was observed after few days after infection. Production of mycelium and spore masses resulting in a salmon coloration was observed in some glumes. Severely infected grains appeared to be shriveled and brittle. Yellow to brownish discolouration was observed on leaves. While, in maize, the first symptoms appeared as necrotic lesions starting from the inoculation point and then expanding through the stem. White to pink cottony mold like growth occurred on kernels of the cob. Thus, it can be concluded from the above observations that *Fusarium* species associated with FHB of wheat might be surviving in the seed and crop residue of cereal crops but not in the soil.

Similar observations were also made by Gordon (1959), Burgess and Griffin (1968) and Warren and Kommedahl (1973) who found that the pathogen, *Fusarium graminearum* survived in old maize stalks as well as ears, stubbles and debris of wheat, barley and other cereal crops. The FHB pathogen has been found to survive as a saprophyte on crop debris and contributes to inoculum for infection of wheat plants in the same field and more distant fields (Keller 2011). Leplat et al. (2013) reported maize to be the more important source of inoculum in locations where both small grain cereals and maize were grown because the debris of latter lasted longer than the former.

4.4.2 Effect of weather variables on disease development

The effect of weather variables viz., temperature, relative humidity and rainfall on the development of FHB on wheat crop sown at three different dates i.e. 31st October, 15th November and 1st December was studied under field conditions. The data on per cent disease severity, maximum and minimum temperature (°C), maximum and minimum RH (%) and rainfall (mm) were recorded after every 10th day starting from the first appearance of the disease.

It is evident from Figure 4.2 that an increase in mean temperature, mean relative humidity and rainfall resulted in gradual increase in the disease severity on 1st, 2nd & 3rd date of sowing, during 2019-20 and 2020-21. The disease severity was more in the 2020-21 as compared to 2019-20 which may be due to more favourable environmental conditions.



The data presented in Table 4.7 indicates that terminal disease severity was higher on 1st December sown crop in comparison to 31st October and 15th November sown crop in both the cropping years i.e. 2019-20 and 2020-21. In 2019-20, the terminal disease severity was 41.23, 34.33 and 28.43 per cent on 1st December, 15th November and 31st October sown crop, respectively. Whereas, in 2020-21, it was 44.56, 36.90 and 33.23 per cent on 1st December, 15th November and 31st October sown crop, respectively. The disease severity (a dependent variable) of three dates of sowing was correlated with weather variables viz., mean temperature, mean relative humidity and rainfall which were independent variables and correlation coefficients (simple, partial and multiple) along with regression equations were derived.

In the cropping year 2019-20, the simple correlation coefficients indicated that the disease severity on wheat crop of three dates of sowing (31st October, 15th November and 1st December), had positive correlation with mean temperature (0.900, 0.859 and 0.936), mean relative humidity (0.863, 0.919 and 0.826) and rainfall (0.855, 0.926 and 0.773). However, it showed significant positive correlation with mean temperature in case of 1st and 3rd DOS, mean relative humidity and rainfall (2nd date of sowing).

In 2020-21 also the simple correlation coefficients showed positive correlation of disease severity with mean temperature (0.955, 0.905 and 0.927), mean relative humidity (0.854, 0.848 and 0.734) and rainfall (0.890, 0.863 and 0.862) for three dates of sowing i.e. 31st October, 15th November and 1st December, respectively. However, it showed significant positive correlation with mean temperature in case of 1st, 2nd and 3rd DOS and rainfall in 1st date of sowing.

Partial correlation coefficients also showed positive correlation of disease severity with mean temperature (0.512, 0.619 and 0.809; 0.193, 0.969 and 0.984), mean relative humidity (0.601, 0.605 and 0.884; 0.645, 0.364 and 0.933) and rainfall (0.832, 0.869 and 0.509; 0.657, 0.952 and 0.782) for three dates of sowing (31st October, 15th November and 1st December) during both the cropping years (2019-20 and 2020-21), respectively.

Thus, positive simple and partial correlation coefficients revealed that an increase in individual independent variable viz., temperature, relative humidity and rainfall resulted in the increase in dependent variable i.e. disease severity.

Table 4.7 Effect of weather parameters on severity of Fusarium head blight under field conditions

Year of experiment	Date of sowing	Disease severity (%)	Correlation coefficient							Coefficient of determination (R ²)	Regression equation
			Simple			Partial			Multiple correlation coefficient (R)		
			DS x MT	DS x MRH	DS x R	DS x MT	DS x MRH	DS x R			
2019-20	31 st Oct	28.43	0.900*	0.863	0.855	0.512	0.601	0.832	0.975	0.950	Y=-79.633+0.923X ₁ +1.041X ₂ + 6.335X ₃
	15 th Nov	34.33	0.859	0.919*	0.926*	0.619	0.605	0.869	0.984	0.968	Y=-82.555+1.688X ₁ +0.812X ₂ +5.166X ₃
	1 st Dec	41.23	0.936*	0.826	0.773	0.809	0.884	0.509	0.986	0.973	Y=-313.398+8.296X ₁ +1.826X ₂ +2.763X ₃
2020-21	31 st Oct	33.23	0.955*	0.854	0.890*	0.193	0.645	0.657	0.977	0.955	Y=-484.909+1.013X ₁ +6.218 X ₂ +2.280X ₃
	15 th Nov	36.90	0.905*	0.848	0.863	0.969	0.364	0.952	0.995	0.990	Y=-116.018+3.313X ₁ +0.800X ₂ +3.404X ₃
	1 st Dec	44.56	0.927*	0.734	0.862	0.984	0.933	0.782	0.996	0.993	Y=-425.794+4.906X ₁ +4.346X ₂ +2.039X ₃

*Significant at 5 per cent level

DS= Per cent disease severity (%)

MT= Mean temperature (°C)

MRH= Mean relative humidity (%)

R= Rainfall (mm)

It is further observed that there existed positive multiple correlations between disease severity and three weather factors (independent variables) for three dates of sowing during both the consecutive years. Moreover, these were found to be highly significant on 31st Oct, 15th Nov and 1st Dec sown crop during 2019-20 and 2020-21. The multiple correlation coefficient was highest for 1st Dec sown crop (0.986 and 0.996) followed by 15th Nov (0.984 and 0.995) sown crop and least (0.975 and 0.977) for 31st Oct sown crop during 2019-20 and 2020-21, respectively.

Similarly, coefficients of determination (R^2) suggest that the highest effect of weather variables on disease severity occurred on 1st Dec sown crop (97.30 and 99.30 %) followed by 15th Nov (96.80 and 99.00 %) and least (95.00 and 95.50 %) in 31st Oct sown crop during two consecutive years, respectively.

The regression coefficients of mean temperature, mean relative humidity and rainfall were observed to be positive for all the three dates of sowing (31st Oct, 15th Nov and 1st Dec) during both the consecutive years. The regression equations for three dates of sowing during 2019-20 indicated that a unit change in mean temperature, mean relative humidity and rainfall changed the disease severity by 0.923, 1.041 & 6.335; 1.688, 0.812 & 5.166 and 8.296, 1.826 & 2.763 per cent while, in 2020-21 by 1.013, 6.218 & 2.280; 3.313, 0.800 & 3.404 and 4.906, 4.346 & 2.039 per cent, respectively.

From the above results it can be concluded that all the three weather variables, individually as well as collectively influenced FHB development in wheat.

The effect of environmental (temperature, relative humidity & rainfall) factors on the disease development has been earlier studied by various workers whose observations are in agreement with the present findings. Sutton (1982) noted that the establishment and development of FHB occurred when warm temperatures were accompanied by periods of 92-94 per cent relative humidity during anthesis stage of wheat crop. Shaner (2003) also found that FHB development was favoured by warm temperatures, high rainfall and relative humidity. Saharan et al. (2004) observed that FHB was more prevalent in Northern India because of warm and humid weather during the months of March and April. Weather factors such as moderately high temperature (15 to 30°C), frequent precipitation, prolonged periods (48–72 hrs) of

high moisture and occurrence of air currents favoured FHB infection in wheat (Lenc 2015).

4.4.3 Incubation and Latent period

The incubation and latent periods of *Fusarium graminearum* were ascertained by noting down the symptom development and sporulation regularly upto 10 days after inoculation. The observations indicated that the initial symptoms on earheads appeared as tan brown lesions on glumes after 7 days of inoculation in potted plants. However, the formation of conidia was observed after 9 days of inoculation on spikes. Thus, the incubation and latent periods of 7 and 9 days respectively were observed on the potted wheat plants under greenhouse conditions.

Sakr (2019) observed the typical symptoms of FHB on spikes after 7 days of inoculation with *Fusarium graminearum* on potted wheat plants. Diamond and Cooke (2003) observed that symptoms on ears appeared after 4 to 7 days of inoculation with *Fusarium culmorum*, *F. avenaceum*, *F. poae* and *Microdochium nivale* in a glasshouse experiment.

4.4.4 Host range

The host range of the pathogen (*Fusarium graminearum*) on five cereal crops viz., rice, maize, barley, rye and oat was ascertained by artificially inoculating their potted plants with the conidial suspension of the fungus. It was observed that all the crops showed characteristics symptoms of the disease (Plate 4.6). The symptoms of the disease appeared after 5 -7 days of inoculation. The typical symptoms on these crops are described in Table 4.8.

Table 4.8 Pathogenic behaviour of *Fusarium graminearum* on cereal hosts

Crop	Reaction	Symptoms	
		Earheads/ panicles	Leaves/stem
Rice	+	Light brown spots which later evolved into irregular brown coloured lesions	-
Barley	+	Brown, chlorotic or water soaked lesions were observed on the outer florets	-
Maize	+	-	necrotic lesions on the stalk
Oat	+	Premature bleaching of spikelets	-
Rye	+	Premature bleaching of spikelets	-



Plate 4.6: Studies on host range of *Fusarium graminearum* causing Fusarium head blight of wheat

Osborne and Stein (2007) reported gramineous crops to be the host of *Fusarium graminearum*. *Fusarium* species (teleomorph *Gibberella*) are distributed worldwide, causing several diseases such as foot rot, seedling blight, *Gibberella* stalk and ear rot of maize and *Gibberella* seedling blight and head scab of small grain (wheat, oats, barley, rye and triticale) cereals (Popovski and Celar 2012; Shude et al. 2020). *F. graminearum* was reported to be associated with head blight of rye (Martinez et al. 2019) and oats (Hautsalo 2020).

4.5 Identification of resistance sources for FHB

The growing of resistant cultivars is recognized as the most practical, effective and economic method of disease management in different crops. In the present study, 200 germplasm lines/ genotypes were evaluated for resistance against FHB of wheat under artificial inoculation conditions in the field during two consecutive years and the data on disease severity and reaction on different genotypes are presented in Tables 4.9 and 4.10.

It is evident from the data that out of 200 tested genotypes, thirteen viz., HPW 275, HPW 301, HPW 320, HPW 373, HPW 399, PW 1901, PW 1915, WH 3742, MLW 1438, MLW 1569, HRWYT 226, 17th DSBWYT 47 and 34th SAWSN 3081 were found to be free from disease. Whereas, 26 genotypes viz., HPW 240, HPW 270, HPW 279, HPW 295, HPW 305, HPW 311, HPW 318, HPW 325, HPW 333, HPW 351, HPW 364, HPW 378, HPW 393, HPW 471, PW 1801, PW 1815, PW 1910, WH 711, WH 1169, MLW 1386, MLW 1410, MLW 1471, HT SBWYT 46, 17th DSBWYT 29, 34th SAWSN 3053 and 34th SAWSN 3137 recorded 1 to 5 per cent disease severity and categorized as resistant. While, disease severity ranging between 5 to 25 per cent was recorded on 54 genotypes (HPW 230, HPW 245, HPW 253, HPW 255, HPW 267, HPW 271, HPW 274, HPW 276, HPW 281, HPW 282, HPW 288, HPW 293, HPW 296, HPW 299, HPW 302, HPW 304, HPW 306, HPW 310, HPW 314, HPW 319, HPW 327, HPW 332, HPW 334, HPW 339, HPW 342, HPW 353, HPW 355, HPW 365, HPW 370, HPW 372, HPW 374, HPW 379, HPW 463, HPW 466, HPW 472, PW 1068, PW 1807, PW 1817, PW 1902, PW 1908, PW 1911, WH 1021, WH 1175, MLW 1298, MLW 1403, MLW 1433, MLW 1449, MLW 1454, VL 829, HT SBWYT 29, IBWSNP 1065, 17th DSBWYT 20, 34th SAWSN

3005 and 34th SAWSN 3104) and categorized as moderately resistant. The remaining genotypes were either moderately susceptible (86) or susceptible (21) to FHB of wheat.

In earlier studies, many Indian wheat cultivars like AKDW 2997-16, DBW 62, PBW 396, PDW 311, UAS 415, UP 2747, UP 2798, VL 926, VL 829 and WH 1021 have been found to be moderately resistant by evaluating nearly 4000 genotypes (Saharan 2020). Huang et al. (2019) observed three new wheat lines L958, L962 and L987 to be moderately resistant to FHB. Shi et al. (2020) found eight Chinese wheat cultivars viz., Minfeng266, Cunmai608, Junda159, Zhengmai162, Fengmai12, LS4607, Pumai1165 and Deyan1603 to be moderately resistant to FHB during both the years (2017 & 2018) of testing. Bentivenga et al. (2021) noted that most of the durum wheat cultivars were susceptible to very susceptible to FHB with the exception of Dylan, Tiziana and Dupri which were moderately susceptible to the disease. Sakr and Al-Attar (2021) evaluated six wheat cultivars against four *Fusarium* species of head blight under field conditions during 2020 and 2021 and found two cultivars viz., Bohoth10 and Cham4 to be moderately resistant to the disease.

Table 4.9 Evaluation of wheat germplasm for resistance against Fusarium head blight under artificially inoculated field conditions

S. No.	Genotype/ variety	Disease severity (%)			Disease reaction
		2019-20	2020-21	mean	
1	HPW 89	53.38	55.87	54.63	S
2	HPW 147	26.39	32.16	29.28	MS
3	HPW 155	51.28	53.37	52.33	S
4	HPW 211	28.46	35.87	32.17	MS
5	HPW 224	32.37	41.85	37.11	MS
6	HPW 228	25.38	33.18	29.28	MS
7	HPW 230	15.38	19.39	17.39	MR
8	HPW 233	29.53	36.21	32.87	MS
9	HPW 236	43.29	48.53	45.91	MS
10	HPW 237	26.61	29.87	28.24	MS
11	HPW 240	3.29	4.48	3.89	R
12	HPW 242	26.38	29.53	27.96	MS
13	HPW 243	34.29	37.29	35.79	MS
14	HPW 245	7.28	19.29	13.29	MR
15	HPW 249	43.27	46.29	44.78	MS
16	HPW 251	45.39	49.16	47.28	MS
17	HPW 252	51.28	52.83	52.06	S

18	HPW 253	13.29	22.19	17.74	MR
19	HPW 254	27.92	30.27	29.10	MS
20	HPW 255	15.28	19.68	17.48	MR
21	HPW 260	27.38	31.29	29.34	MS
22	HPW 265	29.13	30.11	29.62	MS
23	HPW 267	16.28	19.29	17.79	MR
24	HPW 270	2.71	3.29	3.00	R
25	HPW 271	9.26	14.28	11.77	MR
26	HPW 274	12.95	18.23	15.59	MR
27	HPW 275	0.00	0.00	0.00	Free
28	HPW 276	19.45	21.28	20.37	MR
29	HPW 279	1.98	2.29	2.14	R
30	HPW 280	25.18	29.07	27.13	MS
31	HPW 281	6.29	17.29	11.79	MR
32	HPW 282	5.39	16.23	10.81	MR
33	HPW 283	25.06	28.18	26.62	MS
34	HPW 284	27.98	35.97	31.98	MS
35	HPW 285	34.29	38.12	36.21	MS
36	HPW 288	14.36	17.73	16.05	MR
37	HPW 292	27.06	30.98	29.02	MS
38	HPW 293	8.03	14.26	11.15	MR
39	HPW 294	29.45	37.84	33.65	MS
40	HPW 295	2.57	4.62	3.60	R
41	HPW 296	7.38	13.28	10.33	MR
42	HPW 297	30.12	38.92	34.52	MS
43	HPW 298	50.93	52.17	51.55	S
44	HPW 299	13.29	23.57	18.43	MR
45	HPW 301	0.00	0.00	0.00	Free
46	HPW 302	11.83	16.34	14.09	MR
47	HPW 303	26.94	29.51	28.23	MS
48	HPW 304	8.04	20.32	14.18	MR
49	HPW 305	3.29	4.18	3.74	R
50	HPW 306	6.23	9.68	7.96	MR
51	HPW 307	25.04	29.56	27.30	MS
52	HPW 308	29.58	36.81	33.20	MS
53	HPW 309	31.08	36.47	33.78	MS
54	HPW 310	12.34	16.68	14.51	MR
55	HPW 311	2.13	3.65	2.89	R
56	HPW 312	27.74	37.84	32.79	MS
57	HPW 313	22.03	32.36	27.20	MS
58	HPW 314	14.82	19.05	16.94	MR
59	HPW 316	51.91	53.28	52.60	S
60	HPW 317	30.18	36.24	33.21	MS
61	HPW 318	1.96	2.47	2.22	R
62	HPW 319	15.27	23.95	19.61	MR
63	HPW 320	0.00	0.00	0.00	Free
64	HPW 324	28.34	35.72	32.03	MS

65	HPW 325	3.92	4.09	4.01	R
66	HPW 326	31.29	36.28	33.79	MS
67	HPW 327	15.02	23.76	19.39	MR
68	HPW 330	29.35	33.74	31.55	MS
69	HPW 332	19.25	23.56	21.41	MR
70	HPW 333	2.97	3.15	3.06	R
71	HPW 334	9.26	18.23	13.75	MR
72	HPW 335	29.35	32.16	30.76	MS
73	HPW 337	26.39	28.41	27.40	MS
74	HPW 338	34.28	39.41	36.85	MS
75	HPW 339	16.28	22.43	19.36	MR
76	HPW 342	18.32	23.64	20.98	MR
77	HPW 349	50.17	51.48	50.83	S
78	HPW 351	2.56	3.01	2.79	R
79	HPW 353	6.28	8.43	7.36	MR
80	HPW 354	31.27	38.75	35.01	MS
81	HPW 355	12.46	18.34	15.40	MR
82	HPW 360	38.42	43.56	40.99	MS
83	HPW 361	26.17	32.68	29.43	MS
84	HPW 362	35.72	41.28	38.50	MS
85	HPW 364	2.16	2.76	2.46	R
86	HPW 365	7.23	18.93	13.08	MR
87	HPW 367	27.07	36.38	31.73	MS
88	HPW 368	39.81	44.26	42.04	MS
89	HPW 370	6.34	18.26	12.30	MR
90	HPW 372	10.67	19.74	15.21	MR
91	HPW 373	0.00	0.00	0.00	Free
92	HPW 374	21.35	24.81	23.08	MR
93	HPW 377	25.53	36.22	30.88	MS
94	HPW 378	3.27	4.12	3.70	R
95	HPW 379	6.38	9.16	7.77	MR
96	HPW 380	30.16	38.26	34.21	MS
97	HPW 382	32.84	43.28	38.06	MS
98	HPW 393	1.28	3.25	2.27	R
99	HPW 399	0.00	0.00	0.00	Free
100	HPW 440	52.19	53.28	52.74	S
101	HPW 441	25.01	29.56	27.29	MS
102	HPW 463	11.93	15.23	13.58	MR
103	HPW 464	34.64	45.56	40.10	MS
104	HPW 466	12.68	22.48	17.58	MR
105	HPW 467	41.28	48.26	44.77	MS
106	HPW 471	2.38	3.19	2.79	R
107	HPW 472	20.28	24.28	22.28	MR
108	HPW 473	39.27	45.81	42.54	MS
109	PW 1068	6.21	15.74	10.98	MR
110	PW 1076	27.19	33.25	30.22	MS
111	PW 1090	25.02	28.43	26.73	MS

112	PW 1801	3.29	4.13	3.71	R
113	PW 1803	28.16	37.22	32.69	MS
114	PW 1806	52.18	54.25	53.22	S
115	PW 1807	14.37	23.74	19.06	MR
116	PW 1810	27.19	36.29	31.74	MS
117	PW 1814	31.21	39.04	35.13	MS
118	PW 1815	2.15	3.75	2.95	R
119	PW 1816	28.91	38.04	33.48	MS
120	PW 1817	16.52	25.32	20.92	MR
121	PW 1901	0.00	0.00	0.00	Free
122	PW 1902	13.27	20.64	16.96	MR
123	PW 1903	26.34	35.48	30.91	MS
124	PW 1904	27.25	28.63	27.94	MS
125	PW 1905	31.27	38.64	34.96	MS
126	PW 1906	26.51	32.69	29.60	MS
127	PW 1908	16.28	21.96	19.12	MR
128	PW 1909	28.53	31.75	30.14	MS
129	PW 1910	2.17	3.28	2.73	R
130	PW 1911	6.39	19.42	12.91	MR
131	PW 1912	27.64	37.18	32.41	MS
132	PW 1913	26.41	29.86	28.13	MS
133	PW 1915	0.00	0.00	0.00	Free
134	PBW 123	25.69	31.29	28.49	MS
135	PBW 291	33.26	38.72	35.99	MS
136	PBW 550	39.15	43.16	41.16	MS
137	PBW 618	27.53	30.75	29.14	MS
138	PBW 621	36.98	31.26	34.12	MS
139	PBW 644	39.71	46.28	43.00	MS
140	PBW 658	35.67	38.19	36.93	MS
141	PBW 660	51.18	54.39	52.79	S
142	PBW 677	50.12	52.76	51.44	S
143	PBW 681	53.27	54.12	53.70	S
144	PBW 695	51.23	54.89	53.06	S
145	PBW 752	52.65	53.13	52.89	S
146	PBW 3742	38.38	46.54	42.46	MS
147	WH 711	3.27	4.28	3.78	R
148	WH 1021	17.29	22.14	19.71	MR
149	WH 1080	40.32	48.34	44.33	MS
150	WH 1105	34.89	42.46	38.68	MS
151	WH 1129	41.27	44.29	42.78	MS
152	WH 1138	51.27	52.84	52.06	S
153	WH 1156	33.86	38.52	36.19	MS
154	WH 1157	41.28	44.83	43.06	MS
155	WH 1169	2.48	3.65	3.07	R
156	WH 1175	6.29	17.51	11.90	MR
157	WH 3742	0.00	0.00	0.00	Free
158	MLW 1158	50.16	51.24	50.70	S

159	MLW 1298	12.85	15.26	14.06	MR
160	MLW 1299	21.27	29.86	25.57	MS
161	MLW 1386	2.85	3.54	3.20	R
162	MLW 1403	5.34	9.75	7.55	MR
163	MLW 1410	1.87	2.45	2.16	R
164	MLW 1433	7.43	9.12	8.28	MR
165	MLW 1438	0.00	0.00	0.00	Free
166	MLW 1449	6.23	9.65	7.94	MR
167	MLW 1454	7.34	11.29	9.32	MR
168	MLW 1471	2.57	3.48	3.03	R
169	MLW 1569	0.00	0.00	0.00	Free
170	HS 277	50.72	51.23	50.98	S
171	HS 490	34.87	43.82	39.35	MS
172	HS 507	32.57	42.97	37.77	MS
173	HS 542	42.86	47.45	45.16	MS
174	HS 562	30.16	34.27	32.22	MS
175	VL 616	26.37	31.98	29.18	MS
176	VL 829	16.28	20.84	18.57	MR
177	VL 892	50.26	51.38	50.82	S
178	VL 907	51.94	52.56	52.25	S
179	PDW 233	51.86	53.57	52.72	S
180	PDW 314	42.18	48.25	45.22	MS
181	NIDW 295	51.96	52.43	52.20	S
182	DDK 1009	32.77	36.12	34.45	MS
183	GW 1189	38.62	41.29	39.96	MS
184	RAJ 1555	42.38	45.58	43.98	MS
185	RAJ 6560	34.28	46.85	40.57	MS
186	HRWYT 226	0.00	0.00	0.00	Free
187	HT SBWYT 29	5.28	8.62	6.95	MR
188	HT SBWYT 46	3.28	4.29	3.79	R
189	IBWSNP 1065	6.24	11.92	9.08	MR
190	17th DSBWYT 20	14.28	19.63	16.96	MR
191	17th DSBWYT 29	3.18	4.32	3.75	R
192	17th DSBWYT 47	0.00	0.00	0.00	Free
193	34th SAWSN 3001	50.16	51.44	50.80	S
194	34th SAWSN 3005	7.88	16.29	12.09	MR
195	34th SAWSN 3032	31.28	36.54	33.91	MS
196	34th SAWSN 3053	2.18	3.63	2.91	R
197	34th SAWSN 3081	0.00	0.00	0.00	Free
198	34th SAWSN 3084	26.13	28.75	27.44	MS
199	34th SAWSN 3104	13.86	22.43	18.15	MR
200	34th SAWSN 3137	2.16	2.67	2.42	R

R- Resistant, MR- Moderately resistant, MS- Moderately susceptible and S- Susceptible

Table 4.10 Categorization of wheat genotypes on the basis of reaction against Fusarium head blight of wheat

Rating	Spikelet infection (%)	Disease reaction	Genotypes	No. of genotypes
0	0	Free	HPW 275, HPW 301, HPW 320, HPW 373, HPW 399, PW 1901, PW 1915, WH 3742, MLW 1438, MLW 1569, HRWYT 226, 17 th DSBWYT 47 and 34 th SAWSN 3081	13
1	1-5	Resistant (R)	HPW 240, HPW 270, HPW 279, HPW 295, HPW 305, HPW 311, HPW 318, HPW 325, HPW 333, HPW 351, HPW 364, HPW 378, HPW 393, HPW 471, PW 1801, PW 1815, PW 1910, WH 711, WH 1169, MLW 1386, MLW 1410, MLW 1471, HT SBWYT 46, 17 th DSBWYT 29, 34 th SAWSN 3053 and 34 th SAWSN 3137	26
2	5-25	Moderately resistant (MR)	HPW 230, HPW 245, HPW 253, HPW 255, HPW 267, HPW 271, HPW 274, HPW 276, HPW 281, HPW 282, HPW 288, HPW 293, HPW 296, HPW 299, HPW 302, HPW 304, HPW 306, HPW 310, HPW 314, HPW 319, HPW 327, HPW 332, HPW 334, HPW 339, HPW 342, HPW 353, HPW 355, HPW 365, HPW 370, HPW 372, HPW 374, HPW 379, HPW 463, HPW 466, HPW 472, PW 1068, PW 1807, PW 1817, PW 1902, PW 1908, PW 1911, WH 1021, WH 1175, MLW 1298, MLW 1403, MLW 1433, MLW 1449, MLW 1454, VL 829, HT SBWYT 29, IBWSNP 1065, 17 th DSBWYT 20, 34 th SAWSN 3005 and 34 th SAWSN 3104	54

3	25-50	Moderately Susceptible (MS)	HPW 147, HPW 211, HPW 224, HPW 228, HPW 233, HPW 236, HPW 237, HPW 242, HPW 243, HPW 249, HPW 251, HPW 254, HPW 260, HPW 265, HPW 280, HPW 283, HPW 284, HPW 285, HPW 292, HPW 294, HPW 297, HPW 303, HPW 307, HPW 308, HPW 309, HPW 312, HPW 313, HPW 317, HPW 324, HPW 326, HPW 330, HPW 335, HPW 337, HPW 338, HPW 354, HPW 360, HPW 361, HPW 362, HPW 367, HPW 368, HPW 377, HPW 380, HPW 382, HPW 441, HPW 464, HPW 467, HPW 473, PW 1076, PW 1090, PW 1803, PW 1810, PW 1814, PW 1816, PW 1903, PW 1904, PW 1905, PW 1906, PW 1909, PW 1912, PW 1913, PBW 123, PBW 291, PBW 550, PBW 618, PBW 621, PBW 644, PBW 658, PBW 3742, WH 1080, WH 1105, WH 1129, WH 1156, WH 1157, MLW 1299, HS 490, HS 507, HS 542, HS 562, VL 616, PDW 314, DDK 1009, GW 1189, RAJ 1555, RAJ 6560, 34 th SAWSN 3032, 34 th SAWSN 3084	86
4	50-75	Susceptible (S)	HPW 89, HPW 155, HPW 252, HPW 298, HPW 316, HPW 349, HPW 440, PW 1806, PBW 660, PBW 677, PBW 681, PBW 695, PBW 752, WH 1138, MLW 1158, HS 277, VL 892, VL 907, PDW 233, NIDW 295 and 34 th SAWSN 3001	21
5	>75	Very Susceptible (VS)	Nil	0

4.6 Management of the disease

4.6.1 Effect of different sowing dates

Alteration in sowing dates of different crops plays a significant role in the escape of many diseases. The effect of three different sowing dates viz., 31st Oct (early), 15th Nov (timely) and 1st Dec (late) on FHB development was studied by growing artificially inoculated seed of wheat variety HPW 89 in a pot experiment and data on disease incidence and severity were presented in Table 4.11.

Table 4.11 Effect of sowing dates on the development of Fusarium head blight of wheat

Sowing date	Disease incidence (%)	Disease severity (%)
31 st Oct (Early)	27.02	31.83
15 th Nov (Timely)	36.44	42.18
1 st Dec (Late)	50.82	61.15

The data reveal that the highest incidence (50.82%) and severity (61.15%) of FHB occurred on late sown (1st Dec) followed by timely sown (15th Nov) which showed 36.44 and 42.18 per cent of disease incidence and severity, respectively. However, the lowest incidence (27.02%) and severity (31.83%) were noted on the early sown (30th Oct) crop. Hence, the data reveal that early and timely sowing significantly reduced the incidence and severity of FHB in wheat.

The present findings are corroborated by the observations of Arthur (1891) who reported that early sown wheat varieties were more resistant to FHB than the late sown varieties. Jenny et al. (2000) reported that the early sowing facilitated the infection of Fusarium crown rot, whereas late sowing favoured the development of FHB. Subedi et al. (2007) also found that the late planting resulted in more incidence of FHB. Parikka et al. (2008) reported that late-maturing oat lines were more susceptible to FHB than the early-maturing ones.

4.6.2 *In vitro* evaluation of organic and natural preparations/ amendments against *Fusarium graminearum*

Nine organic and natural preparations/ amendments were evaluated *in vitro* for their inhibitory effect against *Fusarium graminearum* and data presented in Table 4.12 and Plate 4.7.

Perusal of the data reveal that Beejamrit @ 50, 40 & 30 per cent and Jeevamrit @ 50 & 40 per cent gave complete inhibition of mycelial growth of *F. graminearum*. These were followed by Vermiwash (50%), Beejamrit (20%), Vermiwash (40%), Vermiwash (30%), Beejamrit (10%), Vermiwash (20%), Jeevamrit (30%), Jeevamrit (20%), Vermiwash (10%), Brahmastra (50%) and Neem cake (50%) giving 85.40, 83.27, 82.64, 78.29, 76.73, 73.19, 71.41, 66.46, 65.45, 63.38 and 52.63 per cent mycelial inhibition, respectively. Rest of the treatments at different concentrations gave less than 50 per cent inhibition and were not much effective. It is thus concluded that Beejamrit was the most effective treatment followed by Jeevamrit and Vermiwash. Whereas, Biosol was the least effective giving minimum inhibition at 20, 30, 40 and 50 per cent concentration as compared to other treatments at these concentrations.

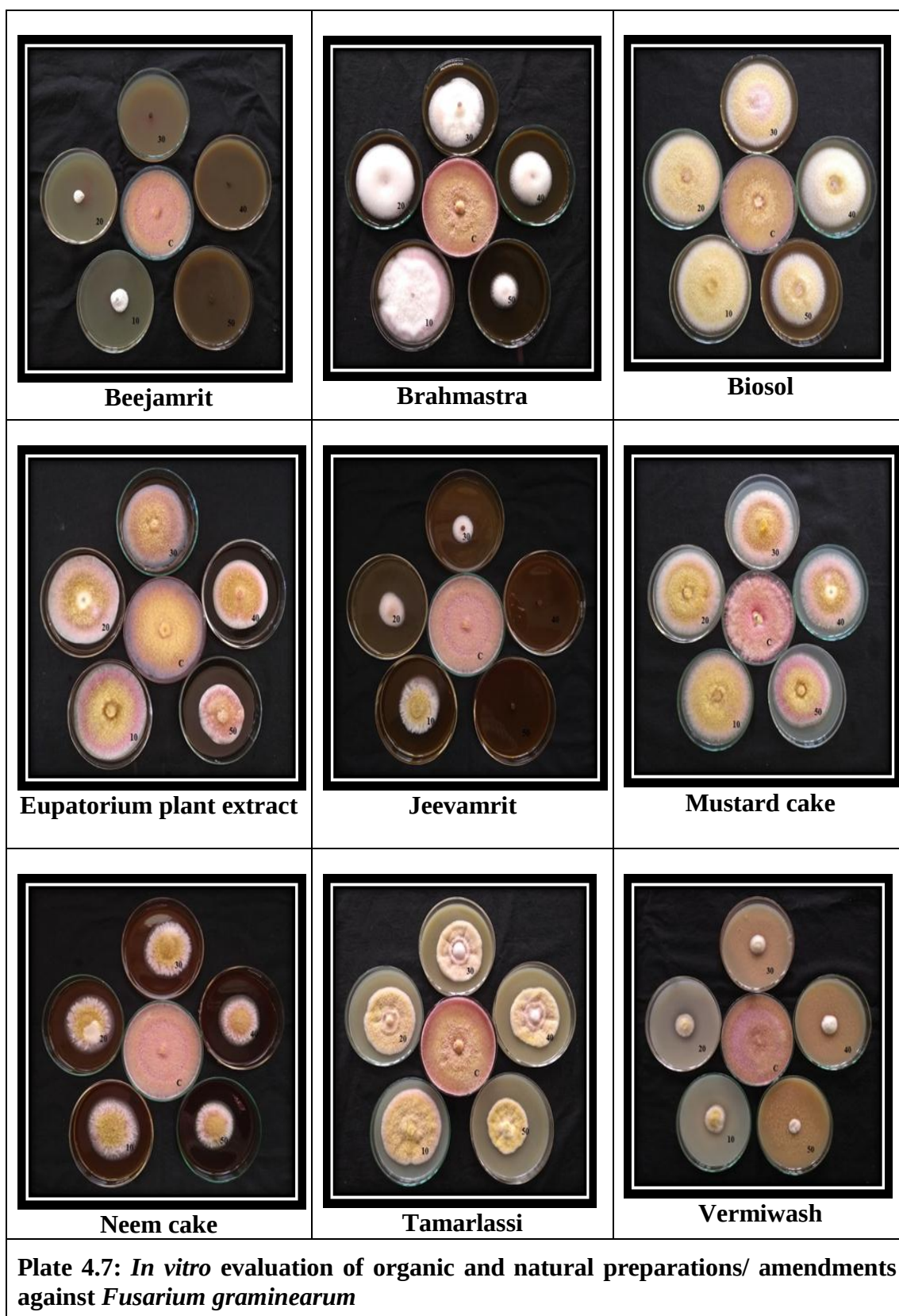
The results obtained in the present investigation are in accordance of Pathania et al. (2019) who reported that at 10 per cent concentration, beejamrit and vermiwash exhibited 59.2 and 38.5 per cent mycelial inhibition of *Fusarium oxysporum* f. sp. *ciceris*, respectively. However, biosol was found least effective with only 24.8 per cent mycelial inhibition over control. Akinnuoye-Adelabu et al. (2019) reported 13.77 ± 2.4 and 10.66 ± 0.7 per cent mycelial growth inhibition of *F. graminearum* with 14 and 7 days old vermiwash, respectively. Rana et al. (2020) reported that vermiwash and beejamrit exhibited 61.11 and 59.63 per cent mycelial inhibition of *Fusarium oxysporum* f. sp. *ciceris*, respectively. The presence of microorganisms (bacteria, fungi and actinomycetes) and their antifungal compounds in organic inputs contributes towards mycelial inhibition (Swaminathan et al. 2007; Ashlesha and Sugha 2008).

Table 4.12 *In vitro* evaluation of organic and natural preparations/ amendments against *Fusarium graminearum*

Organic and natural preparations/ amendments	Mycelial growth (mm) at concentration (%)					Mycelial inhibition (%) at concentration (%)				
	10	20	30	40	50	10	20	30	40	50
Brahmastra	85.01(67.21)*	71.30(57.58)*	66.45(8.21)**	56.10(7.55)**	32.96(5.82)**	5.54	20.78	26.16	37.67	63.38
Eupatorium plant extract	87.20(69.01)	83.65(66.12)	74.26(8.67)	64.15(8.07)	51.52(7.24)	3.11	7.06	17.49	28.72	42.76
Tamarlassi	70.25(56.93)	65.57(54.05)	58.13(7.68)	54.91(7.47)	48.43(7.02)	21.95	27.14	35.41	38.99	46.19
Mustard cake	84.97(67.17)	83.31(65.86)	78.67(8.92)	76.15(8.78)	70.17(8.43)	5.59	7.44	12.59	15.39	22.03
Jeevamrit	55.10(47.90)	30.19(33.31)	25.73(5.16)	0.00(1.00)	0.00(1.00)	38.78	66.46	71.41	100.00	100.00
Vermiwash	31.10(33.87)	24.13(29.40)	19.54(4.53)	15.62(4.07)	13.14(3.76)	65.45	73.19	78.29	82.64	85.40
Beejamrit	20.94(27.19)	15.06(22.82)	0.00(1.00)	0.00(1.00)	0.00(1.00)	76.73	83.27	100.00	100.00	100.00
Neem cake	65.19(53.82)	60.10(50.80)	57.79(7.66)	50.16(7.15)	42.63(6.60)	27.56	33.23	35.79	44.27	52.63
Biosol	86.36(68.30)	85.19(67.34)	82.70(9.14)	79.16(8.93)	77.23(8.84)	4.04	5.34	8.11	12.05	14.19
Control	90.00(71.53)	90.00(71.53)	90.00(9.53)	90.00(9.53)	90.00(9.53)	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	1.92	1.02	0.09	0.12	0.17	-	-	-	-	-

*Figures within parentheses are arc sine transformed values

**Figures within parentheses are square root transformed values



4.6.3 *In vitro* evaluation of botanicals against *Fusarium graminearum*

Naturally occurring biologically active compounds from plants are generally assumed to be more acceptable and less hazardous than synthetic compounds and represent a rich source of potential disease control agents. The presence of antifungal compounds in higher plants has long been recognized as an important factor to disease resistance. Such compounds being biodegradable and selective in their toxicity are considered valuable for controlling different plant diseases.

4.6.3.1 Evaluation of aqueous extracts of botanicals

The aqueous extracts of 11 botanicals were evaluated *in vitro* for their inhibitory effect against *Fusarium graminearum* and data presented in Table 4.13 and Plate 4.8.

The data show that all the aqueous extracts of botanicals at lower concentrations (10 & 20 %) gave less than 50 per cent mycelial growth inhibition. However, aqueous extract of *Acorus calamus* at higher concentrations i.e. 50, 40 and 30 per cent gave 78.73, 65.77 and 60.10 per cent inhibition followed by aqueous extract of *Withania somnifera*, *Vitex negundo* and *Polygonum hydropiper* all at 50 per cent concentration giving 54.73, 52.71 and 50.28 per cent inhibition. Except these none of the botanicals gave more than 50 per cent inhibition at 50 per cent concentration and were not much effective. From these results it is concluded that aqueous extract of *Acorus calamus* $\geq$ 30 per cent concentration was the most effective botanical, whereas, aqueous extract of *Melia azedarach* and *Euphorbia royleana* were least effective at all the concentrations.

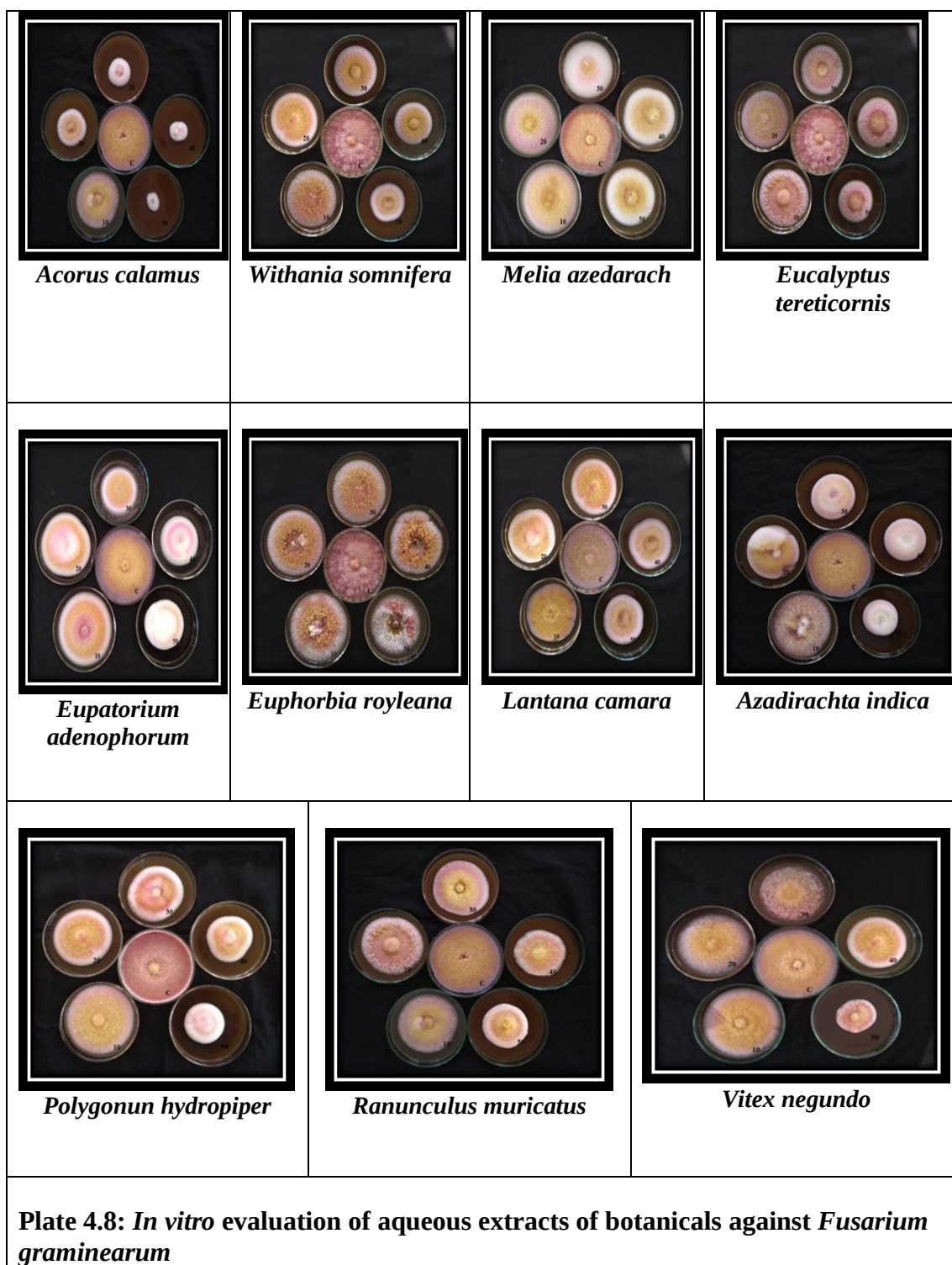
4.6.3.2 Evaluation of alcoholic extracts of botanicals

The alcoholic extracts of 11 botanicals were also evaluated *in vitro* for their inhibitory effect against *Fusarium graminearum* under *in vitro* conditions.

Table 4.13 *In vitro* evaluation of aqueous extracts of botanicals against *Fusarium graminearum*

Botanical	Mycelial growth* (mm) at different concentration (%)					Mycelial inhibition (%) at different concentration (%)				
	10	20	30	40	50	10	20	30	40	50
<i>Acorus calamus</i>	75.25(60.14)	49.78(44.85)	35.91(36.80)	30.81(33.69)	19.14(25.93)	16.39	44.69	60.10	65.77	78.73
<i>Withania somnifera</i>	85.07(67.25)	71.43(57.66)	64.59(53.46)	47.95(43.80)	40.74(39.64)	5.47	20.64	28.23	46.73	54.73
<i>Melia azedarach</i>	86.98(68.82)	82.97(65.60)	80.94(64.09)	75.43(60.26)	73.85(59.21)	3.35	7.81	10.07	16.19	17.95
<i>Eucalyptus tereticornis</i>	85.88(67.89)	81.32(64.36)	69.15(56.23)	64.31(53.29)	52.07(46.17)	4.58	9.64	23.17	28.54	42.14
<i>Eupatorium adenophorum</i>	88.05(69.75)	86.15(68.13)	64.74(53.55)	62.18(52.03)	57.83(49.48)	2.17	4.28	28.06	30.91	35.75
<i>Euphorbia royleana</i>	86.81(69.69)	86.18(68.15)	79.85(63.31)	78.84(62.59)	71.11(57.47)	3.54	4.24	11.28	12.40	20.99
<i>Lantana camara</i>	83.85(66.28)	81.29(64.35)	76.42(60.92)	60.74(51.18)	52.08(46.17)	6.83	9.67	15.09	32.51	42.13
<i>Azadirachta indica</i>	84.92(67.12)	75.63(60.39)	64.16(53.20)	58.11(49.64)	49.11(44.47)	5.64	15.97	28.71	35.43	45.43
<i>Polygonum hydropiper</i>	87.40(69.18)	75.15(60.07)	67.77(55.39)	59.91(50.69)	44.75(41.96)	2.89	16.50	24.70	33.43	50.28
<i>Vitex negundo</i>	87.81(69.55)	85.67(67.72)	76.23(60.80)	65.41(53.95)	42.56(40.70)	2.44	4.81	15.30	27.32	52.71
<i>Ranunculus muricatus</i>	76.92(61.27)	70.18(56.87)	67.44(55.18)	56.41(48.66)	51.63(45.91)	14.53	22.02	25.07	37.32	42.64
Control	90.00(71.53)	90.00(71.53)	90.00(71.53)	90.00(71.53)	90.00(71.53)	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	1.14	1.10	1.24	0.92	1.03	-	-	-	-	-

*Figures within parentheses are arc sine transformed values



The perusal of data given in Table 4.14 and Plate 4.9 indicate that alcoholic extract of *Acorus calamus* was the most effective botanical as its alcoholic extracts at 40 and 50 per cent concentration gave cent per inhibition of mycelial growth. It was the only botanical whose even the lowest concentrations (30, 20 & 10 %) gave 82.88, 63.74 and 54.46 per cent inhibition. It was followed by *Withania somnifera*, *Vitex negundo*, *Polygonum hydropiper*, *Eupatorium adenophorum* and *Lantana camara* whose alcoholic extracts at 50 per cent concentration gave 65.27, 61.83, 60.14, 59.70 and 59.52 per cent inhibition. Alcoholic extracts of *Withania somnifera*, *Vitex negundo* and *Eupatorium adenophorum*, all at 40 per cent concentration, were the next for their efficacy with 55.52, 51.41 and 50.11 per cent inhibition. Rest of the botanicals at different concentration were not much effective as they gave less than 50 per cent mycelial growth inhibition. However, alcoholic extract of *Euphorbia royleana* was the least effective at all concentrations, except 10 and 20 per cent.

The data also reveal that with the increase in the concentration of extracts of different botanicals, there was a corresponding increase in the mycelial inhibition of the pathogen. The alcoholic extracts of these plants were found more effective as compared to aqueous extracts. Among botanicals, aqueous and alcoholic extracts of *Acorus calamus*, *Withania somnifera* and *Vitex negundo* were found more effective. Ghosh (2006) attributed the antifungal properties of *Acorus calamus* to haem peroxidase which is synthesized and accumulated in the plant during pathogenesis. Siva et al. (2008) observed that *Vitex negundo*, at 10 per cent concentration *in vitro* showed the highest inhibitory effect (82%) against *Fusarium* species, followed by *Sapindus emarginatus* (74%) and *Adhatoda vasica* (72%).

Rawal et al. (2015) found that the acetone extract of *Acorus calamus* was most effective *in vitro* against *Fusarium oxysporum* f. sp. *lycopersici* with inhibition zone of 29.5 mm at 1000 mg/ mL concentration, followed by its ethanol and aqueous extracts, with inhibition zone of 19.6 mm and 15.1 mm, respectively. Seepe et al. (2021) noted that the leaf extracts of *Withania somnifera* (L.) Dunal

Table 4.14 *In vitro* evaluation of alcoholic extracts of botanicals against *Fusarium graminearum*

Botanical	Mycelial growth (mm) at concentration (%)					Mycelial inhibition (%) at concentration (%)				
	10	20	30	40	50	10	20	30	40	50
<i>Acorus calamus</i>	40.99(39.79)*	32.63(34.82)*	15.41(23.10)*	0.00(1.00)**	0.00(1.00)**	54.46	63.74	82.88	100.00	100.00
<i>Withania somnifera</i>	80.21(63.57)	62.38(52.14)	55.13(47.92)	40.03(6.40)	31.26(5.68)	10.87	30.69	38.75	55.52	65.27
<i>Melia azedarach</i>	82.30(65.10)	76.30(60.84)	70.29(56.95)	61.83(7.92)	51.65(7.25)	8.56	15.23	21.90	31.30	42.61
<i>Eucalyptus tereticornis</i>	75.03(60.00)	68.76(55.99)	61.39(51.56)	55.70(7.53)	51.55(7.24)	16.63	23.60	31.79	38.11	42.72
<i>Eupatorium adenophorum</i>	86.15(68.14)	82.34(65.12)	64.56(53.44)	44.90(6.77)	36.27(6.10)	4.27	8.51	28.27	50.11	59.70
<i>Euphorbia royleana</i>	84.80(67.03)	78.32(62.22)	71.94(57.99)	65.93(8.18)	63.36(8.02)	5.77	12.98	20.07	26.75	29.60
<i>Lantana camara</i>	88.07(69.80)	71.01(57.40)	57.23(49.13)	54.30(7.43)	36.43(6.11)	2.14	21.10	36.41	39.67	59.52
<i>Azadirachta indica</i>	80.45(63.74)	75.68(60.43)	63.88(53.03)	58.81(7.73)	49.06(7.07)	10.61	15.91	29.03	34.66	45.49
<i>Polygonum hydropiper</i>	86.00(68.00)	76.86(61.22)	71.35(57.61)	59.60(7.78)	35.87(6.07)	4.45	14.60	20.73	33.77	60.14
<i>Vitex negundo</i>	85.13(67.29)	69.39(56.39)	56.10(48.48)	43.73(6.68)	34.36(5.94)	5.41	22.90	37.66	51.41	61.83
<i>Ranunculus muricatus</i>	86.61(68.52)	74.26(59.48)	62.82(52.41)	56.12(7.55)	48.48(7.03)	3.77	17.49	30.20	37.64	46.13
Control	90.00(71.53)	90.00(71.53)	90.00(71.53)	90.00(9.53)	90.00(9.53)	0.00	0.00	0.00	0.00	0.00
CD(p=0.05)	1.67	1.10	0.87	0.12	0.09	-	-	-	-	-

*Figures within parentheses are arc sine transformed value

**Figures within parentheses are square root transformed values



and *Combretum erythrophyllum* (Burch.) Sond. exhibited good antifungal activity against one or more of the tested *Fusarium* pathogens (*F. graminearum*, *F. oxysporum*, *F. subglutinans*, *F. semitectum*, *F. verticilloides*, *F. proliferatum*, *F. solani* and *F. chlamydosporum*) with minimum inhibitory concentrations less than 1.0 mg/ mL. Dinev et al. (2021) revealed that *Acorus calamus* extracts showed the highest antimicrobial activity against eight fungal strains (*Fusarium graminearum*, *Aspergillus flavus*, *F. oxysporum*, *A. parasiticus*, *Penicillium chrysogenum*, *A. ochraceus*, *Alternaria alternata*, *A. niger* and *A. carbonarius*), followed by the *Chlorella vulgaris*, *Lemna minuta* and *Scenedesmus dimorphus* extracts, which provided inhibition of two to three strains. They also observed that *S. dimorphus* extract exhibited the highest antioxidant potential, followed by *A. calamus* and *L. minuta* methanolic extracts, however *C. vulgaris* extracts exhibited lowest antioxidant activity.

4.6.4 In vitro evaluation of biocontrol agents against *Fusarium graminearum*

The use of biological control agents in plant disease management has become an area of intensive research in view of the hazardous impact of agrochemicals on ecosystem and human/ animal health.

The present study was conducted to determine the antagonistic effect of a few fungi and a bacterium on the growth of *Fusarium graminearum*. The data in respect of mycelial growth and per cent inhibition of the fungus are presented in Table 4.15, Plate 4.10 and Figure 4.3. It is evident from the data that all the tested bioagents significantly affected the mycelial growth of *F. graminearum*. However, *Trichoderma harzianum* (TH-5) was found to be the most effective with 43.64 per cent mycelial growth inhibition of the pathogen over the control. The pathogen showed least mycelial growth of 44.34 mm in comparison to the control having 78.67 mm mycelial growth. It was followed by *T. koningii* (DMA-8) (37.95%), *T. viride* (Tv) (37.09%), *T. koningii* (JMA-11) (34.62%), *Trichoderma* spp. (T-3) (30.73%), *Trichoderma* spp. (T-6) (28.27%), *Pseudomonas fluorescens* (22.47%), *Trichoderma* spp. (T-5) (22.06%), *Trichoderma* spp. (T-2) (21.17%) and *Trichoderma* spp. (T-4) (19.13%). While, *Trichoderma* spp. (T-1) was observed to be least effective with 69.14 mm

mycelial growth and 12.11 per cent growth inhibition. None of the biocontrol agents gave ≥ 50 per cent inhibition over the control.

Table 4.15 *In vitro* evaluation of biocontrol agents against *Fusarium graminearum*

Biocontrol agent	Average mycelial growth* (mm)	Per cent growth inhibition over control (%)
<i>Trichoderma koningii</i> (JMA-11)	51.43(45.80)	34.62
<i>Trichoderma harzianum</i> (TH-5)	44.34(41.72)	43.64
<i>Trichoderma koningii</i> (DMA-8)	48.81(44.30)	37.95
<i>Trichoderma viride</i> (Tv)	49.50(44.69)	37.09
<i>Trichoderma</i> spp. (T-1)	69.14(56.23)	12.11
<i>Trichoderma</i> spp. (T-2)	62.02(51.93)	21.17
<i>Trichoderma</i> spp. (T-3)	54.50(47.56)	30.73
<i>Trichoderma</i> spp. (T-4)	63.63(52.89)	19.13
<i>Trichoderma</i> spp. (T-5)	61.32(51.52)	22.06
<i>Trichoderma</i> spp. (T-6)	56.43(48.67)	28.27
<i>Pseudomonas fluorescens</i>	61.00(51.33)	22.47
Control	78.67(62.62)	0.00
CD(p=0.05)	3.01	-

*Figures within parentheses are arc sine transformed values

The possible mechanisms of antagonism employed by *Trichoderma* spp. include antibiosis by producing volatile and non-volatile antibiotics, nutrient and niche competition and mycoparasitism which are inhibitory against a range of phytopathogenic fungi (Dennis and Webster 1971; Harman and Hadar 1983; Schirmbock et al. 1994). Stimulation of host defence responses is another possible mechanism of biocontrol activity of *Trichoderma* spp. (Howell et al. 2000). Hasan et al. (2012) found that *T. harzianum* inhibited the radial growth of *F. graminearum* by 43.33 per cent under laboratory conditions. Mahmoud (2016) observed that seven isolates of *T. harzianum* inhibited the mycelial growth with the inhibition rate of 51, 49, 65, 63, 60, 46 and 48 per cent respectively under *in vitro* conditions. Gupta (2019) found that *T. harzianum* (TH-5) was highly effective against *F. graminearum* as it resulted in 40.33 per cent mycelial inhibition in dual culture whereas, *T. harzianum* (TH-11) was least effective.

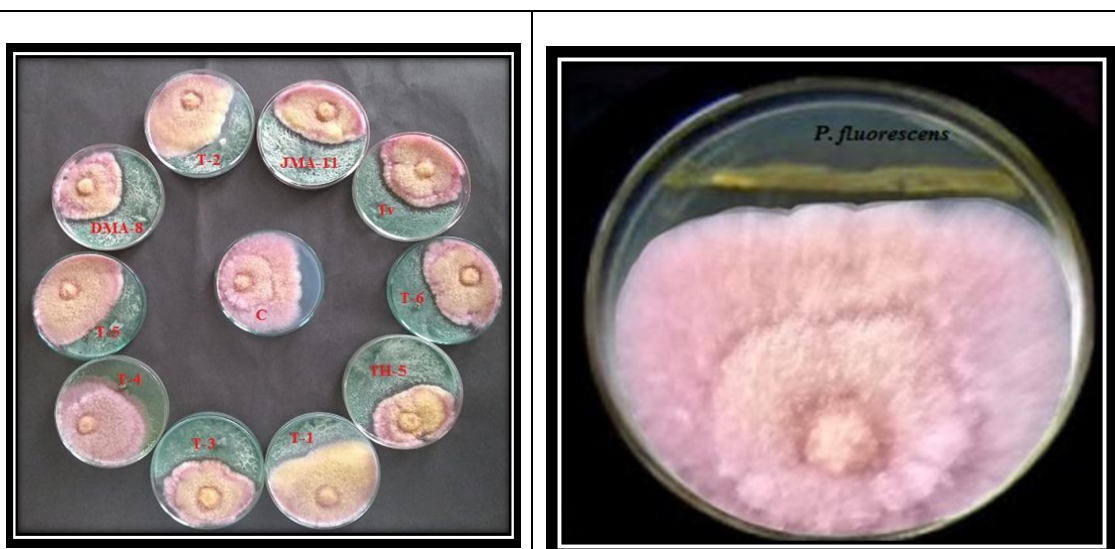


Plate 4.10: *In vitro* evaluation of biocontrol agents against *Fusarium graminearum*

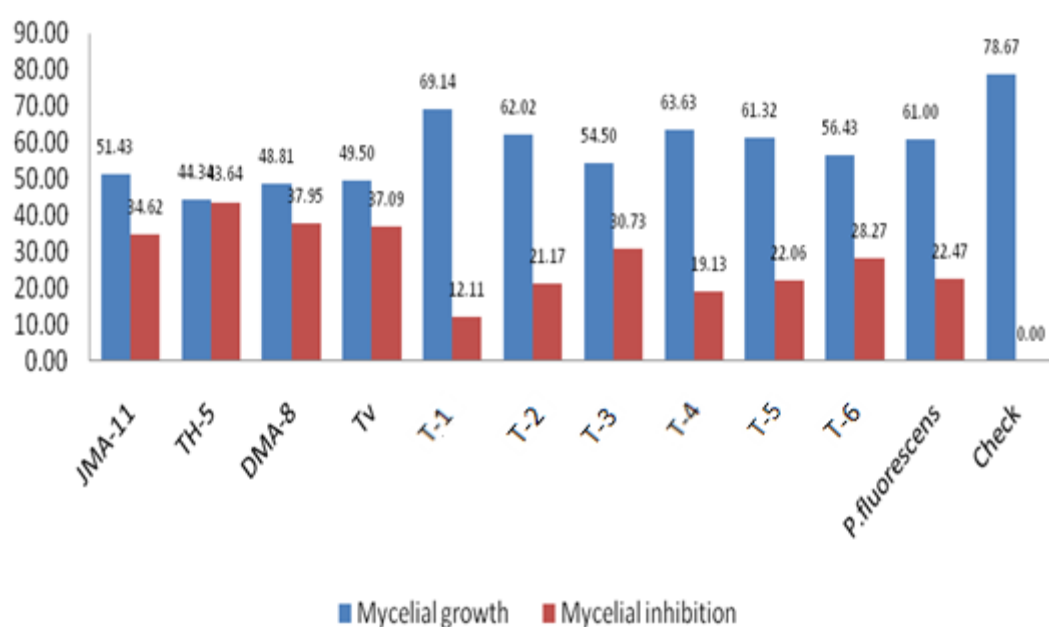


Figure 4.3 Mycelial growth (mm) and inhibition (%) of *Fusarium graminearum* by different bioagents

4.6.5 *In vitro* evaluation of fungicides against *Fusarium graminearum*

Chemical control of plant diseases with fungicides is one of the oldest and discrete method of disease management. Nine fungicides including systemic, non-systemic and combination formulations were evaluated against *F. graminearum* at different concentrations (50 to 2000 ppm) *in vitro* and data on mycelial growth and per cent inhibition are shown in Table 4.16 and Plate 4.11.

It is exhibited by the data that all the fungicides and combination formulations except copper oxychloride gave complete inhibition of mycelial growth at ≥ 500 ppm concentrations. At 250 ppm, propiconazole 25 EC, tebuconazole 250 EC, carbendazim 50 WP, trifloxystrobin 25% + tebuconazole 50% WG and carboxin 37.5% +thiram 37.5% WS all gave cent per cent inhibition and followed by difenaconazole 25 EC, carbendazim 12% + mancozeb 63% WP and mancozeb 75WP with 84.28, 78.52 and 56.30 per cent inhibition. At 100 ppm concentration, tebuconazole 250 EC, carbendazim 50 WP and trifloxystrobin 25% + tebuconazole 50% WG gave complete inhibition and followed by carboxin 37.5% +thiram 37.5% WS, propiconazole 25 EC, difenaconazole 25 EC and carbendazim 12% + mancozeb 63% WP with 88.15, 85.46, 77.24 and 72.18 per cent mycelial inhibition. At 50 ppm carbendazim 50 WP gave the maximum inhibition of 87.77 per cent followed by trifloxystrobin 25% + tebuconazole 50% WG, tebuconazole 250 EC, carboxin 37.5% + thiram 37.5% WS and propiconazole 25 EC with 85.33, 83.84, 80.74 and 77.41 per cent inhibition. Copper oxychloride 50 WP, a non-systemic fungicide was found effective only at higher concentrations i.e. 2000, 1500, 1000 and 750 ppm giving 90.96, 80.01, 71.16 and 61.23 per cent mycelial inhibition.

From the above results it is concluded that carbendazim 50 WP, trifloxystrobin 25% + tebuconazole 50% WG and tebuconazole 250 EC were the most effective fungicides against *F. graminearum* while, Copper oxychloride 50 WP was the least effective one.

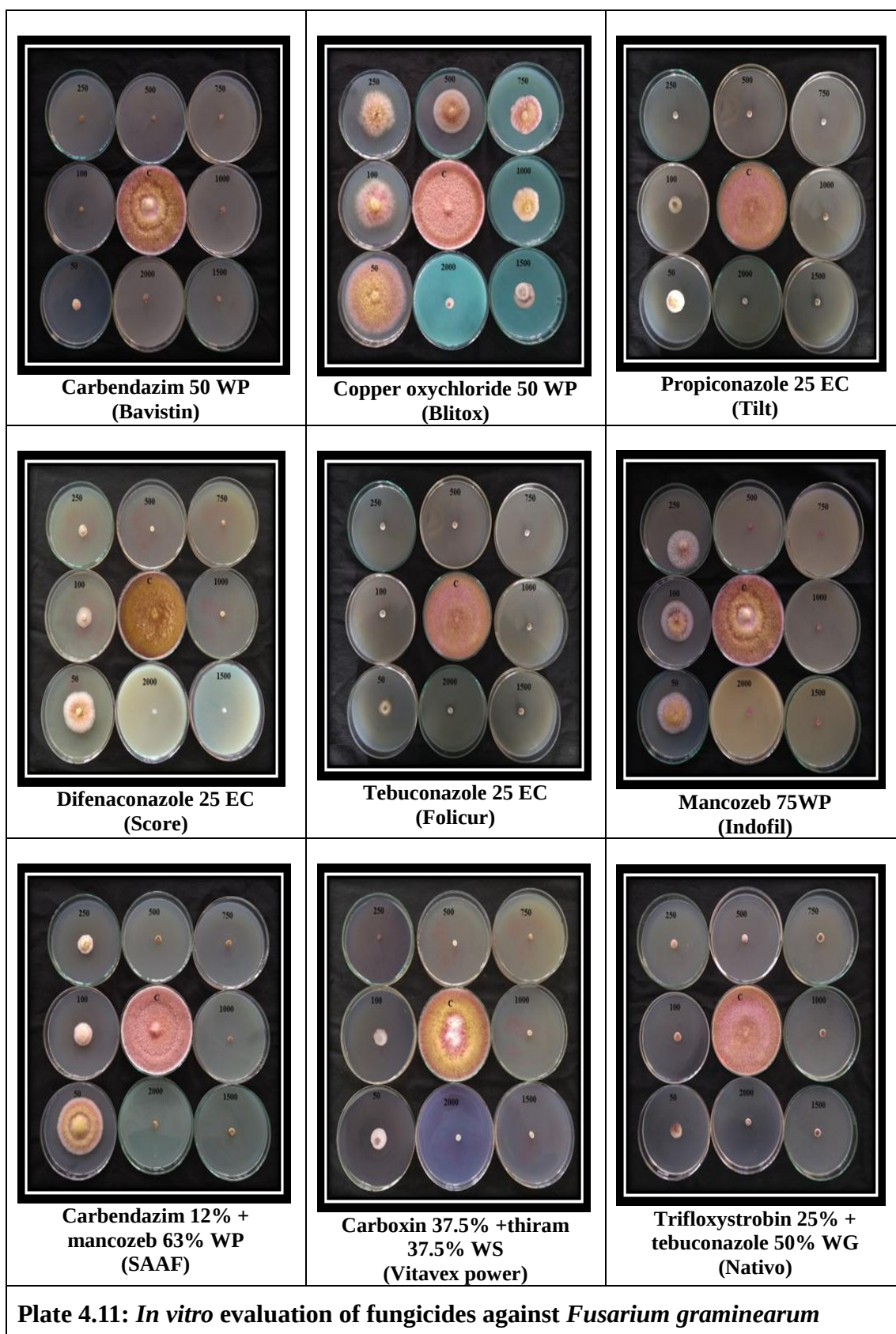
The effectiveness of some of the above tested fungicides has also been reported by earlier workers (Boshoff et al. 1999; Ivic et al. 2011; Avozani et al. 2014). Jones (2000) reported that *F. graminearum* isolates were sensitive to benomyl, tebuconazole, fludioxonil and mancozeb at concentrations of 3.5×10^{-1} , 9.9×10^{-1} ,

Table 4.16 *In-vitro* evaluation of fungicides against *Fusarium graminearum*

Fungicide	Mycelial growth (mm) at different concentration (µg/ mL)								Mycelial inhibition (%) at different concentration (µg/ mL)							
	50	100	250	500	750	1000	1500	2000	50	100	250	500	750	1000	1500	2000
Difenaconazole 25 EC (Score)	44.23(41.66)*	20.48(4.63)**	14.15(3.87)**	0.00(1.00)**	0.00(1.00)**	0.00(1.00)**	0.00(1.00)**	0.00(1.00)**	50.86	77.24	84.28	100.00	100.00	100.00	100.00	100.00
Propiconazole 25 EC (Tilt)	20.33(26.78)	13.09(3.75)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	77.41	85.46	100.00	100.00	100.00	100.00	100.00	100.00
Tebuconazole 25 EC (Folicur)	14.55(22.39)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	83.84	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Carbendazim 50 WP (Bavistin)	11.01(19.35)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	87.77	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Trifloxystrobin 25% + tebuconazole 50% WG (Nativo)	13.21(21.28)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	85.33	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mancozeb 75WP (Indofil)	51.11(45.61)	45.62(6.82)	39.33(6.35)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	43.21	49.31	56.30	100.00	100.00	100.00	100.00	100.00
Carbendazim 12% + mancozeb 63% WP (SAAF)	53.33(46.89)	25.04(5.10)	19.33(4.50)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	40.74	72.18	78.52	100.00	100.00	100.00	100.00	100.00
Carboxin 37.5% +thiram 37.5% WS (Vitavax power)	17.33(24.59)	10.67(3.41)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	0.00(1.00)	80.74	88.15	100.00	100.00	100.00	100.00	100.00	100.00
Copper oxychloride 50 WP (Blitox)	81.33(64.37)	60.78(7.86)	50.67(7.18)	45.25(6.79)	34.89(5.98)	25.96(5.18)	17.45(4.29)	8.14(3.01)	9.63	32.47	43.70	49.73	61.23	71.16	80.61	90.96
Control	90.00(71.53)	90.00(9.53)	90.00(9.53)	90.00(9.53)	90.00(9.53)	90.00(9.53)	90.00(9.53)	90.00(9.53)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CD (p=0.05)	1.28	0.13	0.12	0.11	0.13	0.10	0.12	0.10	-	-	-	-	-	-	-	-

*Figures within parentheses are arc sine transformed value

**Figures within parentheses are square root transformed values



7.4×10^{-3} and 7.3 mg a.i./ L, respectively under laboratory conditions. Mullenborn et al. (2008) recorded high sensitivity of different *Fusarium* spp. viz., *F. graminearum*, *F. culmorum* and *F. poae* to tebuconazole and prothioconazole as compared to fluoxastrobin and azoxystrobin under laboratory conditions. Gupta (2019) found that Vitavax power and Bavistin showed cent per cent mycelial inhibition of *F. graminearum* even at 50 ppm concentration, whereas tilt was found least effective (55.74% inhibition) at the same concentration.

4.6.6 In vivo evaluation of organic and natural preparations/ amendments, botanicals, biocontrol agents and fungicides against Fusarium head blight

Three highly effective treatments of each management component under *in vitro* studies were further evaluated as seed treatment and foliar sprays against FHB in a pot experiment and data recorded on disease incidence and severity are tabulated in Table 4.17. The data reveal that among organic preparations, beejamrit gave maximum reduction of 53.65 and 58.26 per cent in disease incidence and severity followed by jeevamrit (50.73 & 55.79 %) and vermiwash (49.26 & 54.89 %), respectively. In case of bio control agents, *Trichoderma harzianum* (TH-5) was most effective with 41.95 and 43.79 per cent reduction in disease incidence and severity, respectively. This was followed by *T. koningii* (DMA-8) and *T. viride* (Tv) which provided 39.07 & 40.72 and 35.38 & 39.21 per cent reduction in disease incidence and severity, respectively. Among botanicals, aqueous extract of *Acorus calamus* gave the highest reduction of 47.24 and 52.36 per cent in disease incidence and severity, respectively. Whereas, *Withania somnifera* and *Vitex negundo* extracts exhibited 45.79 & 49.75 and 44.68 & 46.23 per cent reduction in disease incidence and severity, respectively.

Amongst three fungicides, carbendazim 50 WP gave maximum reduction of 71.85 and 77.16 per cent in disease incidence and severity, respectively. It was followed by trifloxystrobin 25% + tebuconazole 50% WG and tebuconazole 250 EC which provided 67.23 & 74.26 and 65.53 & 70.30 per cent reduction in disease incidence and severity, respectively.

It is further revealed by data that among all the disease management components, fungicides were highly effective and carbendazim 50 WP gave maximum reduction in incidence and severity of FHB over the check. However, among non-chemical components all the three organic/ natural preparations (which were statistically at par) and aqueous extract of *Acorus calamus* were comparatively more effective giving more than 50 per cent reduction in disease severity.

Table 4.17 *In vivo* evaluation of potential organic preparations, biocontrol agents, botanicals and fungicides against Fusarium head blight of wheat

Treatment	Disease incidence (%)	Reduction in disease incidence (%)	Disease severity (%)	Reduction in disease severity (%)
Jeevamrit	25.41(30.23)*	50.73	28.19(32.03) *	55.79
Vermiwash	26.18(30.75)	49.26	28.76(32.40)	54.89
Beejamrit	23.91(29.25)	53.65	26.61(30.99)	58.26
<i>Trichoderma koningii</i> (DMA-8)	31.43(34.05)	39.07	37.79(37.91)	40.72
<i>Trichoderma harzianum</i> (TH-5)	29.94(33.15)	41.95	35.83(36.73)	43.79
<i>Trichoderma viride</i> (Tv)	33.33(35.23)	35.38	38.76(38.48)	39.21
<i>Withania somnifera</i>	27.97(31.88)	45.79	32.03(34.45)	49.75
<i>Vitex negundo</i>	28.54(32.27)	44.68	34.28(35.81)	46.23
<i>Acorus calamus</i>	27.22(31.43)	47.24	30.37(33.41)	52.36
Tebuconazole 250 EC	17.78(24.82)	65.53	18.94(25.78)	70.30
Carbendazim 50 WP	14.52(22.33)	71.85	14.56(22.37)	77.16
Trifloxystrobin 25% + tebuconazole 50% WG	16.91(24.15)	67.23	16.41(23.85)	74.26
Control	53.01(45.89)	0.00	63.75(52.96)	0.00
CD(p=0.05)	3.24	-	2.07	-

*Figures within parentheses are arc sine transformed values

The results obtained in the present investigation are in accordance with the findings of Cromeey et al. (2001) who found that tebuconazole and carbendazim provided substantial reduction in both FHB incidence and mycotoxins levels while azoxystrobin was ineffective in doing so. Riungu et al. (2008) observed that folicur gave maximum reduction in disease severity (47 %) of FHB followed by copper oxychloride (36 %) under greenhouse conditions.

Mahmoud (2016) noted that *T. harzianum* gave a significant reduction (55.0 %) in head blight severity while *B. subtilis* showed just 36.7 per cent reduction under greenhouse conditions. Machado et al. (2017) reported that triazoles (tebuconazole, prothioconazole and metconazole) and benzimidazoles were the most effective fungicides for controlling FHB of wheat.

4.6.7 Integrated management of FHB

The present study on the integration of different disease management for the management of FHB of wheat using infected seed of susceptible variety HPW 89 under field conditions during *Rabi* 2020-21. The data on terminal disease incidence and severity were recorded after 10 days of 3rd (final) spray and presented in Table 4.18. The data indicate that the minimum disease incidence (13.57 %) and severity (15.25 %) occurred in case of treatment T1 i.e. seed treatment with carbendazim 50 WP (2 g/ kg seed) followed by its two foliar sprays (1 g/ L water) at 10 days interval. It resulted into maximum reduction in disease incidence (62.65%) and severity (66.48 %) over the check. However, data on the integration of disease management components showed that treatment T7 i.e. seed treatment with carbendazim 50 WP (2 g/ kg seed) followed by one foliar spray each of Jeevamrit (10 mL/ L water), aqueous extract of *Acorus calamus* (10 mL/ L water) and carbendazim 50 WP (1 g/ L water) at 10 days interval resulted in 57.26 and 61.56 per cent reduction in disease incidence and severity, respectively. It was followed by treatment T4 i.e. seed treatment with carbendazim 50 WP (2 g/ kg seed) followed by one foliar spray each of Jeevamrit (10 mL/ L water) and aqueous extract of *Acorus calamus* (10 mL/ L water) at 10 days interval giving 53.05 and 56.08 per cent reduction in disease incidence and severity, respectively. Whereas, the least reduction in disease incidence (24.02 %) and severity (30.55 %) was given by treatment T2 i.e. seed treatment with *Trichoderma harzianum* (10 g/ kg seed) followed by two foliar sprays of *Acorus calamus* (10 mL/ L water), at 10 days interval.

Table 4.18 Evaluation of integrated treatments against Fusarium head blight under field conditions

Treatment	Disease incidence (%)	Reduction in disease incidence (%)	Disease severity (%)	Reduction in disease severity (%)	AUDPC	Rate of disease increase (r)
T1: Seed treatment with carbendazim 50 WP @ 2 g/ kg seed + two foliar sprays of carbendazim 50 WP @ 1 g/ L water	13.57(21.58)*	62.65	15.25(22.97)*	66.48	404.23	0.19
T2: Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/ kg seed + two foliar sprays of <i>Acorus calamus</i> @ 10 mL/ L water	27.60(31.66)	24.02	31.60(34.17)	30.55	679.47	0.46
T3: Seed treatment with Beejamrit @ 10 mL/ kg seed + two foliar sprays of Jeevamrit @ 10 mL/ L water	25.96(30.61)	28.53	29.88(33.10)	34.34	665.12	0.42
T4: Seed treatment with carbendazim 50 WP @ 2 g/ kg seed + foliar spray of Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water	17.05(24.29)	53.05	19.98(26.53)	56.08	517.80	0.25
T5: Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/ kg seed + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water	23.72(29.10)	34.70	27.40(31.52)	39.79	640.77	0.41
T6: Seed treatment with Beejamrit @ 10 mL/ kg seed + foliar spray of <i>Acorus calamus</i> @ 10 mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water	20.78(27.05)	42.80	24.19(29.43)	46.84	595.62	0.30
T7: Seed treatment with carbendazim 50 WP @ 2 g/ kg seed + foliar spray of Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water	15.52(23.13)	57.26	17.49(24.70)	61.56	431.67	0.20
T8: Seed treatment with <i>Trichoderma harzianum</i> @ 10 g/ kg seed+ foliar spray of Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @ 1 g/ L water	21.51(27.59)	40.77	25.79(30.48)	43.32	625.47	0.34
T9: Seed treatment with Beejamrit @ 10 mL/ kg seed + foliar spray of Jeevamrit @ 10 mL/ L water + foliar spray of <i>Acorus calamus</i> @ 10mL/ L water + foliar spray of carbendazim 50 WP @1 g/ L water	19.56(26.22)	46.14	22.77(28.48)	49.97	574.72	0.28
T10: Check	36.32(37.03)	0.00	45.50(42.40)	0.00	805.30	0.64
CD(p=0.05)	1.80	-	1.23	-	28.02	0.05

*Figures within parentheses are arc sine transformed values

The values of area under disease progress curve (AUDPC) for different treatments indicate that seed treatment with carbendazim 50 WP (2 g/ kg seed) followed by its two foliar sprays (1 g/ L water) resulted in minimum AUDPC value (404.23), followed by seed treatment with carbendazim 50 WP (2 g/ kg seed) followed by one foliar spray each of Jeevamrit (10 mL/ L water), aqueous extract of *Acorus calamus* (10 mL/ L water) and carbendazim 50 WP (1 g/ L water) with AUDPC value of 431.67. However, the maximum AUDPC value (679.47) was observed for treatment T2 i.e. seed treatment with *Trichoderma harzianum* (10 g/ kg seed) followed by two foliar sprays of *Acorus calamus* (10 mL/ L water).

The data on rate of disease increase (r) reveal that its value ranged between 0.19 to 0.46 for different treatments. It was maximum (0.46) for treatment T2 i.e. seed treatment with *Trichoderma harzianum* (10 g/ kg seed) followed by two foliar sprays of *Acorus calamus* (10 mL/ L water).

However, the minimum value of 'r' (0.19) was recorded in case of treatment T1 i.e. seed treatment with carbendazim 50 WP (2 g/ kg seed) followed by its two foliar sprays (1 g/ L water).

It is thus clear from the above results that seed treatment and foliar sprays of carbendazim 50 WP alone or its combinations with other management components is the most effective way to manage this disease.

For the effective and ecofriendly management of diseases, it is always recommended that fungicides should be used in combination with other control strategies such as biological control and resistant cultivars. In several disease management strategies, the addition of resistance inducing chemical in combination with biocontrol agent has significantly enhanced the disease control, compared to treatments with biocontrol agent alone (Frances et al. 2002; Buck and Jeffers 2004). De Waard et al. (1993) reported that fungicide application had a better impact when it was used within an integrated disease management strategy. The probable reasons for variable effects of fungicides against FHB might be cultivar resistance, fungicide efficacy, fungicide coverage, timing and frequency of applications, different epidemiology and the natural variation in aggressiveness of individual pathogens (Homdork et al. 2000). Mesterhazy et al. (2003) found that the fungicide efficacy in controlling FHB and DON accumulation was higher in the more resistant than in the

more susceptible winter wheat cultivars. Yin et al. (2009) reported carbendazim to be more effective against *F. culmorum*, *F. avenaceum* and *F. graminearum*. Bleakley et al. (2012) reported that use of fungicides alone or in combination with biological control agents (BCAs) resulted in reduced yield losses. Thus, farmers in areas where FHB is more likely to occur are encouraged to integrate as many management components as possible.

5. SUMMARY AND CONCLUSIONS

The present investigation on “**Epidemiology and management of Fusarium head blight of wheat**” was undertaken with the objectives of studying the role of epidemiological factors in disease development, identification of resistance sources, evaluation of disease management components and their integration. The significant findings are summarized below:

Fusarium head blight (FHB) of wheat was prevalent at all the surveyed locations of the main wheat growing districts of the state during two consecutive years i.e. 2019-20 and 2020-21. The maximum average disease incidence and severity were observed in district Una (7.74 & 25.29 %) followed by Kangra (5.94 & 24.19 %) and Bilaspur (5.89 & 20.71 %), respectively. Amongst the individual locations, maximum mean disease incidence and severity were recorded at Duhki (12.22 & 33.85 %) of district Una followed by Malan (10.77 & 31.34 %) of district Kangra and Gehrwin (9.35 & 27.78 %) of district Bilaspur, respectively. Three *Fusarium* species viz., *Fusarium graminearum*, *F. compactum* and *F. avenaceum* were isolated from infected spikes and found pathogenic on wheat. However, *F. graminearum* was more prevalent in all the surveyed locations of the state.

The temperature had a significant effect on the *in vitro* growth of *F. graminearum*. The maximum mycelial growth (90.00 mm) was recorded at 25°C followed by 30°C (87.60 mm) and 35°C (48.00 mm) after 7 days of incubation whereas, minimum growth (31.67 mm) was observed at 10°C. The highest sporulation (5.5×10^6 conidia/ mL) and high perithecia formation were observed at 25°C followed by 30°C with sporulation of 5.2×10^6 per mL and less perithecia formation. However, the least sporulation (1.1×10^6 conidia/ mL) and very less perithecia formation were observed at 10°C.

A pH level of 5.0 was found to be most suitable giving maximum mycelial growth (86.00 mm), sporulation (5.7×10^6 conidia/ mL) and perithecia formation of the fungus after 7 days of incubation followed by 6.0 and 7.0. Whereas, the minimum

growth (30.33 mm) was observed at pH 8 and least sporulation (1.9×10^6 conidia/ mL) and perithecia formation occurred at pH 4.

Fusarium species isolated from the seed and crop residue of wheat, maize and rice were found to be pathogenic on all the three cereal crops. While, *Fusarium* species isolated from the soil of wheat, maize and rice fields were non pathogenic on any of the crops. Thus, infected seed and crop residue were the main source of inoculum.

The effect of weather parameters on disease development revealed that an increase in mean temperature, mean relative humidity and rainfall resulted in gradual increase in the disease severity on 1st, 2nd and 3rd date of sowing, during 2019-2020 and 2020-2021. The terminal disease severity was higher on late (1st December) sown crop (41.23 & 44.56 %) in comparison to early (31st October) (28.43 & 33.23 %) and timely (15th November) (34.33 & 36.90 %) sown crops in both the cropping years, respectively.

The simple, partial and multiple correlation coefficients indicated positive correlations between disease severity and weather variables (mean temperature, mean relative humidity and rainfall) individually as well as collectively for all the three sowing dates during two consecutive years. The coefficients of determination (R^2) indicated that the highest effect of weather variables on disease severity occurred on late (1st Dec) sown crop followed by timely (15th Nov) and least on early (31st Oct) sown crop during both the cropping years. The regression equations between disease severity and weather variables showed that a unit increase in individual weather variable directly increased the disease severity (by the respective values of the weather variables) in the same direction, in crops of all the sowing dates during both the years.

The initial symptoms of the disease on earheads appeared after 7 days of inoculation while, the formation of conidia took place after 9 days of inoculation, indicating the incubation and latent periods to be 7 and 9 days respectively under greenhouse conditions.

Fusarium graminearum isolated from wheat earheads showed its host range to rice, maize, barley, rye and oat crops.

Thirteen genotypes (HPW 275, HPW 301, HPW 320, HPW 373, HPW 399, PW 1901, PW 1915, WH 3742, MLW 1438, MLW 1569, HRWYT 226, 17th DSBWYT 47 and 34th SAWSN 3081) were found to be free from disease, while 26 (HPW 240, HPW 270, HPW 279, HPW 295, HPW 305, HPW 311, HPW 318, HPW 325, HPW 333, HPW 351, HPW 364, HPW 378, HPW 393, HPW 471, PW 1801, PW 1815, PW 1910, WH 711, WH 1169, MLW 1386, MLW 1410, MLW 1471, HT SBWYT 46, 17th DSBWYT 29, 34th SAWSN 3053 and 34th SAWSN 3137) were resistant to FHB.

Amongst three dates of crop sowing viz., early (31st Oct), timely (15st Nov) and late (1st Dec), the maximum disease incidence (50.82 %) and severity (61.15 %) were recorded in late sown condition. Whereas, the minimum disease incidence (27.02 %) and severity (31.83 %) were noted on the early sown (30th Oct) crop.

In vitro testing of nine organic and natural preparations/ amendments (Vermiwash, Beejamrit, Jeevamrit, Brahmastra, Tamarlassi, Eupatorium plant extract, Biosol, Neem and Mustard cake) showed Beejamrit to be highly effective giving complete mycelial growth inhibition of *F. graminearum* at ≥ 30 per cent concentrations whereas, Biosol was found least effective (14.19 % inhibition) at 50 per cent concentration.

Among aqueous extracts of 11 botanicals (*Azadirachta indica*, *Melia azedarach*, *Eucalyptus tereticornis*, *Eupatorium adenophorum*, *Ranunculus muricatus*, *Euphorbia royleana*, *Vitex negundo*, *Lantana camara*, *Polygonum hydropiper*, *Withania somnifera* and *Acorus calamus*), *Acorus calamus* at 50 per cent concentration was most effective with 78.73 per cent mycelial growth inhibition of *F. graminearum in vitro* while, *Melia azedarach* was found least effective (17.95 % inhibition) at the same concentration.

Amongst alcoholic extracts of above mentioned botanicals, *Acorus calamus* at ≥ 40 per cent concentrations gave complete inhibition of *F. graminearum* whereas, *Euphorbia royleana* showed least mycelial inhibition (29.60 %) at 50 per cent concentration.

The *in vitro* evaluation of eleven bioagents revealed *Trichoderma harzianum* (TH-5) to be the most effective giving 43.64 per cent mycelial growth inhibition of

the pathogen while, *Trichoderma* spp. (T-1) was least effective with 12.11 per cent growth inhibition.

Among nine fungicides evaluated *in vitro* against *F. graminearum*, carbendazim 50 WP, trifloxystrobin 25% + tebuconazole 50% WG and tebuconazole 250 EC at ≥ 100 ppm concentrations were most effective showing complete inhibition of mycelial growth.

Under *in vivo* conditions, seed treatment with carbendazim 50 WP followed by its two foliar sprays gave maximum reduction (71.85 and 77.16 %) in disease incidence and severity, respectively. However, among non-chemical approaches seed treatment with beejamrit, *Acorus calamus* extract and *T. harzianum* (TH-5) followed by two foliar sprays of each, individually resulted in 53.65 & 58.26, 47.24 & 52.36 and 41.95 & 43.79 per cent reduction in disease incidence and severity, respectively.

Under field conditions, seed treatment with carbendazim 50 WP (0.2 %) and its two foliar sprays (0.1 %) at 10 days interval gave highest (62.65 & 66.48 %) reduction in disease incidence and severity, respectively. However, among integrated treatments, seed treatment with carbendazim 50 WP (0.2 %) and three foliar sprays each of Jeevamrit (1 %), aqueous extract of *Acorus calamus* (1 %) and carbendazim 50 WP (0.1 %) at 10 days interval was found to be most effective giving 57.26 and 61.56 per cent reduction in disease incidence and severity, respectively.

Conclusions:

- FHB was prevalent at all the surveyed locations of Himachal Pradesh and its average incidence and severity varied from 3.25 to 7.74 and 15.08 to 25.29 per cent during 2019-20 and 2020-21, respectively. The highest disease incidence and severity were recorded in district Una followed by Kangra and Bilaspur districts during both the years.
- *Fusarium graminearum*, *F. compactum* and *F. avenaceum* were found associated with the disease but *F. graminearum* was more prevalent in all the surveyed locations of Himachal Pradesh.
- The optimum temperature and pH for the growth, sporulation and perithecia formation of *F. graminearum* were 25°C and pH 5.0.
- The seed and crop residue of maize, wheat and rice were confirmed to be the primary source of inoculum for the disease development.
- All the weather factors (temperature, relative humidity and rainfall) contributed positively in the development of the disease.
- Cereal crops viz., maize, rice, rye, barley and oat were found to be the hosts of *Fusarium graminearum*.
- Thirteen genotypes of wheat were found to be free from disease while, 26 were resistant.
- The early and timely sowing of wheat were found to be the best as they resulted in considerable reduction in disease incidence and severity in comparison to late sowing.
- Under *in vitro* conditions, botanical *Acorus calamus* (aqueous and alcoholic) extract, natural preparation beejamrit, bioagent *Trichoderma harzianum* (TH-5) and fungicide carbendazim 50 WP gave highest reduction in mycelial growth of the fungus.
- Under *in vivo* conditions, *Acorus calamus* extract, beejamrit, *Trichoderma harzianum* (TH-5) and carbendazim 50 WP individually were found most

effective, giving highest reduction in disease incidence and severity of the disease.

- Seed treatment with carbendazim 50 WP followed by its two foliar sprays gave maximum reduction in the incidence and severity of the disease under field conditions.
- The integrated treatment, seed treatment with carbendazim 50 WP followed by three foliar sprays each of Jeevamrit, *Acorus calamus* extract and carbendazim 50 WP was found most effective in managing the disease.

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APPENDICES

APPENDIX-I : Composition of media used

Composition of Potato dextrose agar (PDA)

Peeled potato	200 g
Dextrose	20 g
Agar	20 g
Distilled Water	1000 mL

Composition of Water agar (WA)

Agar	20 g
Distilled Water	1000 mL

APPENDIX-II: Composition of organic inputs

Biosol

Vermicompost	90 kg
Cow dung	75 kg
Cow urine	10 L
Agnihotra solution	250 g ash dissolved in 200 L water
Biodigestor	1000 L capacity
Shri Yantra	1

Vermiwash

Earthen pot	3
15-20 days old cow dung	as per requirement
Easily degradable farm waste	as per requirement
Earthworms	100-200
Water	as per requirement

Beejamrit

Cow dung	10 kg
Cow urine	10 L
Slaked lime	50 g
Soil	Handful
Water	200 L

Jeevamrit

Cow dung	10 kg
Cow urine	10 L
Jaggery	1 kg
Gram flour	1 kg
Soil	Handful
Water	200 L

Brahmastra

Cow dung	10 kg
Cow urine	20 L
Neem leaves and stems	2 kg
Castor leaves and stems	2 kg
Mango leaves and stems	2 kg
Indian Beech leaves and stems	2 kg

Tamarlassi

Butter milk	10 L
Copper vessel	1 no.

Use after 7 days and always prepare fresh

APPENDIX-III: Pedigree of wheat genotypes

S. No.	Genotype/ variety	Pedigree
1	HPW 89	BW 144 INTERMEDIO RODS/HD 2248
2	HPW 147	CPAN 1869/Him 10-BA (WS)
3	HPW 155	BT 2549/Fath (PW 360)
4	HPW 211	MO 88/MILAN
5	HPW 224	WW 24/ Lehmi P ₁
6	HPW 228	262# 106K99-245
7	HPW 230	HPW 42/IBWSN-52
8	HPW 233	RL-6-3-1/K8504 P2
9	HPW 236	-

10	HPW 237	WW 24/Lehmi (P1)
11	HPW 240	-
12	HPW 242	-
13	HPW 243	CPAN 1796/CPAN 1922
14	HPW 245	WW 24/Lehmi (P2)
15	HPW 249	691P1-U-132
16	HPW 251	WW24/LEHMIP2-U149
17	HPW 252	WW 24/ Lehmi 677-P1-U-33-P1
18	HPW 253	PRENIA CM.90722-22Y-OM-OY-SM-OY-OSY-OHTY
19	HPW 254	OPATA/RAYON//KAUZ
20	HPW 255	WW 24/ Lehmi –P3-U-132
21	HPW 260	CHH 80A542/CNO.79 CMH83
22	HPW 265	F 81513/ MILAN-CMSS 96Y02706 S-040Y-020 M-050Y-52 M-0Y
23	HPW 267	HS 382/ WL 711
24	HPW 270	ATTILA * 2/ PASTOR
25	HPW 271	C 564 P ₆ P ₄
26	HPW 274	TURACO/ PRINIA
27	HPW 275	CPAN 4029/ PBW 51
28	HPW 276	-
29	HPW 279	SERI* 3/ RL 6010/ 4* YR/ 3/ PASTOR/ 4/ BAV 92
30	HPW 280	SHA 7/ VEE# 5/ 5/ VEE# 8// JUP/ BJY/ 3/ F ₃ / F ₃ .71/ TRM/ 4/-----
31	HPW 281	PW 351/ HPW 89-P ₁
32	HPW 282	OTUS-TOBA 97
33	HPW 283	CROC-1AE SQUARROSA (205)///KAUZ/ 3/ SASIA
34	HPW 284	OTUS/ TOBA 97
35	HPW 285	CROC-AE SQUARROSA (205)// KAUZ/ 3/ SASIA
36	HPW 288	WW 10/WW 24

37	HPW 292	TRAP/1/ BOW// PFAV/ 3/ MILAN
38	HPW 293	VHW 3574 DWR-WS 18-1
39	HPW 294	SITE/ MO/ 4/ NAC/ TH. AC// 3* PVN/ 3/ MIRLO/ BOC
40	HPW 295	CPAN 3004/ HS 295
41	HPW 296	-
42	HPW 297	-
43	HPW 298	RDWG/ MILAN
44	HPW 299	VHW 3574 DWR-WS 18-2
45	HPW 301	W 462// VEE/ KOEL/3 PEG// MRL/ BOC
46	HPW 302	SNI/ 2* PASTOR
47	HPW 303	VHW 3574 DWR WS 18-3
48	HPW 304	VHW 3583 DWR WS 24-6
49	HPW 305	VHW 3582 DWR WS 24-8
50	HPW 306	WL 711/ HPW 89
51	HPW 307	NAC/ TH. AC// 3* PVN/ MIRLO/ BUC/ 4/ 2* PASTOR
52	HPW 308	KEHAN 10*2/ 3/ OASIS/ SKAUZ//4*BCN
53	HPW 309	KEHAN *2/ 3/ OASIS/ SKAUZ//4*BCN
54	HPW 310	NAC/ TH. AC// 3* MIRLO/ BUC/ 4/ 2* PASTOR
55	HPW 311	ATTILA* 2/ AMAD
56	HPW 312	CHOIX/ STAR/ 3/ HEI/ 3* CNO 79/ 2 SEN
57	HPW 313	CMH 82 A. 1294/ 2* KAUZ/ MUNIA/ CATO/ 3/ MILAN
58	HPW 314	LONG-291* 2/ PASTOR
59	HPW 316	ACIRNICS 98-3/ HS 295
60	HPW 317	WEAVER// VEE/ RIN/ 3/ MILAN
61	HPW 318	BOW/URES//KEA/3/SITE
62	HPW 319	-
63	HPW 320	41 IBWSN 13 (Augument trial (07-08) NBPGR

64	HPW 324	41 IBWSN 455 (Augument trial (07-08) NBPGR- YCSN (11-12) & NGSN (12-13)
65	HPW 325	41 IBWSN 126 (Augument trial (07-08) NBPGR
66	HPW 326	41 IBWSN 448 (Augument trial (07-08) NBPGR
67	HPW 327	EBWYT 518 (Dr Joshi) (Augument trial (07-08) NBPGR- YCSN (11-12) & NGSN (12-13)
68	HPW 330	12 th SAWYT-35 (2005-06) (C.Block 07-08)
69	HPW 332	ME 11-Q 03-59 (2005-06) EIGN
70	HPW 333	EIGN M1 YCSN-19 (2007-08) EIGN
71	HPW 334	EIGN M1 YCSN-31 (2007-08) EIGN
72	HPW 335	1 st STEMRRSN-6051 EIGN
73	HPW 337	39 th IBWSN-1082 EIGN
74	HPW 338	METSO/ 3/ CROC1/ AE.SQ (213)// PGO
75	HPW 339	PASTOR/ TILHI
76	HPW 342	ATTILA/ 3*BCN*2// BAV 92
77	HPW 349	OASIS/ SKAUZ//4* BCN/ 3/ PASTOR/ 4/ KAUZ-----
78	HPW 351	VHW 3574 DWR-WS 18-4 P ₂
79	HPW 353	ZCL/ 3/ PGFN// CNO 67/ SN 64/ 4/ SERI---
80	HPW 354	-
81	HPW 355	CMH 79. 1384/ 4/ AGA/ 3 SN 64/ CNO 67// INIA 66/ 5----(M1 CSN 16)
82	HPW 360	WL 711/HPW 89
83	HPW 361	WW 10/ WW 24
84	HPW 362	AC 8588/WBLL 1
85	HPW 364	OTUS/KKTS//KKTS
86	HPW 365	CH01X/STAR/3/HEI/3*CNO79/2 SERI
87	HPW 367	55 OASIS/SKAUZ//4*BCN/3/2*PASTOR

88	HPW 368	NAC/TH.AC//3*PVN/3/MIRLO/BUC/4/2*PASTOR
89	HPW 370	MILAN/S 87230//BABAX
90	HPW 372	-
91	HPW 373	MILAN//BUC/CHRC/3/METSO
92	HPW 374	HPW 89/HD 2766 P1
93	HPW 377	HUW 468/HS 295
94	HPW 378	CAR/KAL/BB/3/NAC/4/VEE/PW//2*TUI/5/MILAN
95	HPW 379	VL 804/HPW 42-P2
96	HPW 380	OASIS/SKAUZ//4*BCN*2/3/PASTOR
97	HPW 382	HPW 89/ VL 801
98	HPW 393	VL804/HPW42
99	HPW 399	MILAN/S87230//BABAX
100	HPW 440	VL 858/Tyari 1
101	HPW 441	NAC/TH.AC//3*MIRLO/BUC/4/PASTOR
102	HPW 463	HPW 155/ HW 4024 (P6)
103	HPW 464	Raj3765/WR251//HW 2045/PBW 493
104	HPW 466	PASTOR/H×L 7573/2*BAU/3/SOKOLL/WBLL1
105	HPW 467	HPW 155/ VL 864
106	HPW 471	HPW 236/ VL 900
107	HPW 472	HPW 155/ HD 29
108	HPW 473	HPW 155/ PBW 486
109	PW 1068	NAC/TH.AC//3*MIRLO/BUC/4/2*PASTOR
110	PW 1076	PASTOR/H/L7573/2*BAU/3/SOKOLL/WBLL1
111	PW 1090	VL 832/PBW 498
112	PW 1801	NAC/TH.AC//3*MIRLO/BUC/4/2*PASTOR
113	PW 1803	Long-291 *2/PASTOR
114	PW 1806	ECAR/CHAPIO CMSS99Y014055-040Y-040M-030Y-030M-25Y-

		2M-0Y
115	PW 1807	VL 858/Tyari 1
116	PW 1810	EC 582389/ IC 12037
117	PW 1814	HPW 155/HW 4024-P1
118	PW 1815	HPW 147/ EC 479378
119	PW 1816	HPW 147/ PHS 0628
120	PW 1817	HPW 155/ VL 864
121	PW 1901	(NIAW 34/ CHILERO)F5× PBW 677
122	PW 1902	NAC/TH.AC113*MIRLO/BUC/4/2*PASTOR
123	PW 1903	HPW 155/ HW 4024-P12
124	PW 1904	PFAU/SERI.1B//AMAD/3/WAXWING/4/BABA/x/LR42/BABA x*2/3/KURUKU
125	PW 1905	Sunco/2*Pastor
126	PW 1906	VL 907/IC 296470
127	PW 1908	HS 420/IC 296442
128	PW 1909	HS 490/ WH 423/ HS 490
129	PW 1910	HS 490/ SGP 3
130	PW 1911	BOW/URES//KEA/3/SITE
131	PW 1912	AC 8528/ WBL-1-2
132	PW 1913	VL 616/WW 24
133	PW 1915	Raj 3765/WR251//HW 2045/PBW 493
134	PBW 123	-
135	PBW 291	-
136	PBW 550	-
137	PBW 618	-
138	PBW 621	-
139	PBW 644	PBW175/HD2643

140	PBW 658	-
141	PBW 660	WG6761/WG6798
142	PBW 677	PFAU/MILAN/5/CHEN/ <i>Ae.squarrosa</i> //BCN/3/VEE#7/BOW/4/PASTOR
143	PBW 681	UP2338/KALYANSONA
144	PBW 695	PSN/BOW//MILAN/3/2*BERKUT
145	PBW 752	-
146	PBW 3742	-
147	WH 711	-
148	WH 1021	NYOT95/SONAK
149	WH 1080	PRL/2*PASTOR
150	WH 1105	MILAN/S87230//BABAX
151	WH 1129	CS/TH.CS//3*PVN/3/MIRLO/BUC/4/MILAN/5/TILHI
152	WH 1138	PBW65/2*PASTOR
153	WH 1156	TILHI/PASTOR
154	WH 1157	MUNIA/CHTO//AMSEL
155	WH 1169	-
156	WH 1175	-
157	WH 3742	-
158	MLW 1158	HPW 251/ Lr 17
159	MLW 1298	HS 490 /WR 95
160	MLW 1299	HS 490 / WH 423
161	MLW 1386	HPW 299 / IC 549902
162	MLW 1403	HPW 230 / IC 408332
163	MLW 1410	HPW 89/HPW 211/ HPW 89
164	MLW 1433	HS 490 / YCSN 64
165	MLW 1438	-

166	MLW 1449	-
167	MLW 1454	-
168	MLW 1471	-
169	MLW 1569	HPW 336 /HD 2932
170	HS 277	KAUZ/CGN
171	HS 490	HS364/HPW114//HS240//HS346
172	HS 507	KAUZ/MYNA/VUL//BUC/FLK/4/MILAN
173	HS 542	MILAN/KAUZ//PRINIA/3/BABAX
174	HS 562	OASIS/SKAUZ//4*BCN/3/2*PASTOR
175	VL 616	-
176	VL 829	IBWSN149/CPAN2099
177	VL 892	WH542/PBW226
178	VL 907	DYBR 1982-83842ABVD50/VW9365//PBW343
179	PDW 233	-
180	PDW 314	AJAIA12/F3LOCAL(SEL.ETHIO135.85)//PLATA13/3/SOMAI#3/ 4/SOOTY9/ RASCON37
181	NIDW 295	BOOMER33/PLATA8
182	DDK 1009	-
183	GW 1189	-
184	RAJ 1555	-
185	RAJ 6560	-
186	HRWYT 226	WBLL1/KUKUNA//TACUPETO F2001/6/PVN//CAR422/ANA/5/ BOW/ CROW//BUC/PVN/3/YR/4/TRAP#1/7/WAXWING/KIRITATI*2// YANAC
187	HT SBWYT 29	-
188	HT SBWYT	-

	46	
189	IBWSNP 1065	TACUPETO F2001/BRAMBLING/5/NAC/TH.AC//3*PVN/3/MIRLO/ BUC/4/2*PASTOR*2/6/WAXWING/SRTU//WAXWING/KIRITA TI
190	17th DSBWYT 20	-
191	17th DSBWYT 29	-
192	17th DSBWYT 47	-
193	34th SAWSN 3001	PRL/2*PASTOR
194	34th SAWSN 3005	MUNAL #1
195	34th SAWSN 3032	WAXWING/7/TNMU/6/CEP80111/CEP81165/5/IAC5/4/YKT406/ 3/AG/ASN// ATR/8/ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV9 2
196	34th SAWSN 3053	NELOKI*2/PRL
197	34th SAWSN 3081	WHEAR/KUKUNA/3/C80.1/3*BATAVIA//2*WBLL1*2/6/WBLL 1*2/4/ YACO/PBW65/3/KAUZ*2/TRAP//KAUZ/5/KACHU #1
198	34th SAWSN 3084	VEE#8//JUP/BJY/3/F3.71/TRM/4/BCN/5/KAUZ/6/MILAN/KAUZ/ 7/SKAUZ/ PARUS//PARUS/8/2*TUKURU//BAV92/RAYON/6/NG8201/KAU Z/4/SHA7 //PRL/VEE#6/3/FASAN/5/MILAN/KAUZ

199	34th SAWSN 3104	MUNAL #1/3/KINGBIRD #1//INQALAB 91*2/TUKURU
200	34th SAWSN 3137	FRANCOLIN #1/3/PBW343*2/KUKUNA*2//YANAC/4/KINGBIRD #1 //INQALAB 91*2/TUKURU

Brief Biodata of student

Name : Gurpreet Kaur
Father's Name : Kewal Singh
Mother's Name : Sukhwinder Kaur
Date of Birth : 2nd October, 1995
Permanent Address : Village- Gullarwala karsoli, P.O.- Kalibari,
 Tehsil- Nalagarh, District- Solan, Himachal
 Pradesh, India- 174101

Academic Qualifications:

Qualification	Year	School/Board/University	Marks (%)	Division
10 th	2010	Central Board of Secondary Education (C.B.S.E)	70.3	First
10+2	2012	Central Board of Secondary Education (C.B.S.E)	75.8	First
B.Sc. Agriculture	2016	Punjabi University, Patiala	77.0	First
M.Sc. Plant Pathology	2018	CSK HPKV, Palampur (HP)	77.9	First
Ph.D. Plant Pathology	2022	CSK HPKV, Palampur (HP)	80.5	First

Fellow/Scholarship/Gold Medals/Awards/Any Other Distinction: College stipend

Publications:

1. Total Research papers (in peered journals): - 3
2. Scientific Popular Articles: - 2

Visit abroad along with duration and purpose of visit: NIL