CHAPTER 5

BIOCIDAL ACTIVITY OF ORGANOTIN(IV) COMPLEXES ON FOLIAR BLIGHT DISEASES OF WHEAT (*Triticum aestivum* L.)

5.1 Introduction

India is one of the main wheat producing and consuming countries of the world. After the advent of Green Revolution, the production of wheat has shown a gigantic increase from 10.4 to 80.58 million tones during 2008-09 [1]. The crop is known to suffer from a large number (at least 50 fungal, 7 bacterial and 36 viral) of diseases [2] which may reduces the yield up to 24.3%. Among the different pathogens, foliar blights have been recognized as one of the major production constraint of worldwide wheat cultivation, particularly in warmer growing areas of eastern plains of South Asia including India characterized by an average temperature in the coolest month above 17°C [3-9].

There are numerous foliar blights either of seed borne and/or soil borne diseases reported on wheat [10, 11]. The three blight diseases (spot blotch, tan spot and Alternaria blight) have been recorded in most of the wheat growing areas of India [12-14], Bangladesh [15], Nepal [16] and Pakistan [17]. In Eastern India, the leaf blight diseases represent a complex and are collectively referred to as Helminthosporium Leaf Blight (HLB). Two of the most common diseases, spot blotch and tan spot, are caused by the fungi *B. sorokiniana* (Sacc. in Sorok.) Shoem (Fig. 5.1a); and *Pyrenophora tritici-repentis* (Died) Drechs, respectively. Another leaf blight fungus is *Alternaria triticina* (Fig. 5.1b) reported by Prasad and Prabhu [18]. In the conducive weather conditions i.e. continuous rain for 5-6 day followed by warmer temperatures (day average of 20–30°C), spot blotch epidemic can develop very rapidly [19]. In recent surveys, the relative frequency of *A. triticina* seems to be declining, possibly due to availability of more resistant varieties of wheat [20]. Besides, another leaf spot disease, zonate eye-spot (*Drechslera gigantea*) has also been reported by Chowdhury *et al.* [21] from *terai* zone of West Bengal. Symptoms of these three leaf blights are difficult to distinguish in the field, even microscopic observation does not clearly resolves the problem of correct diagnosis. However, the spot blotch pathogen, *B. sorokiniana* is the major one and responsible for yield loss in northern districts of West Bengal [22]. Though organotin has been widely used as fungicide [23-25], however, till today no attempts have been made to evaluate the oragnotin or its derivatives as fungicide against wheat foliar diseases caused by *B. sorokiniana*, the role of which are elaborated below.

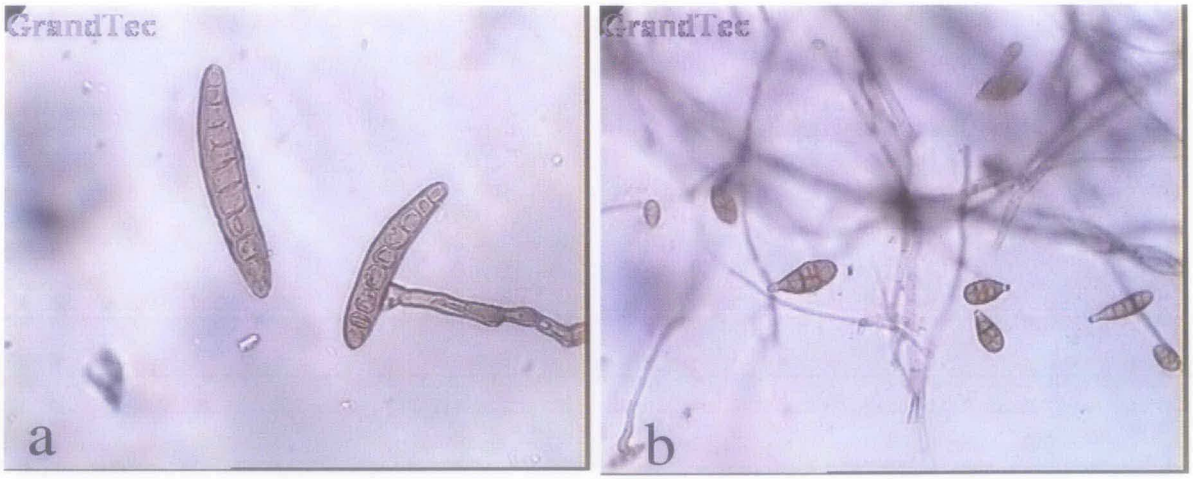


Fig. 5.1 a) Spore of the fungi *B. sorokiniana* and; b) Spore of the fungi *A. triticina*.

5.2 Literature

5.2.1 Use of organotin as biocide

Investigation of fungicidal and bacteriocidal properties of organotin compound and its derivates was carried out by many scientists world over. Among them a few are cited below. In case of the agricultural applications since the present investigation is carried out in Indian soil, similar important works are referred below preferentially. Fentin acetate was found to be effective against many diseases such as leaf spot diseases of bitter gourd [26], leaf spot and fruit rot diseases of brinjal [27], *Phytophthora* diseases of cacao [28], gray blight diseases of coconut [29], sugar cane downy mildew diseases of maize [30], Alternaria disease of sesame [31], kernel bunt [32] and spot blotch diseases of wheat [33]. Similarly, fentin hydroxide was also found to be effective against some diseases of cereal [34-36]. Several other compounds such as fentin chloride [37], non-commercialized triphenyl tin derivatives [38, 39] and some anionic complexes [40] were found to be effective as fungicide or as bactericide. Kamruddin *et al.* [41] have reported that among the three $R_3SnO_2(O_2CCH_2N(H)C(O)NH_2)$ [R=Ph,C-Hex(Cyclohexyl) or n-Bu], n-Bu was more toxic against the fungi *A. alternate*, *H. sativum*, *H. maydis* and *P. oryzae*. Chakraborty *et al.* [42] have reported the synthesis and biocidal activity of $[R_3Sn(O_2CCH_2SC_5H_4N$-4] where R=Ph, benzyl(Bz), Cyclohexyl(C-hex), n-Bu and $[R_3Sn\{O_2CCH_2SC_4H_3N_2$-2,6] where R=Me, Ph and n-Bu against the fungi *H. maydis* (ITCC 2675) and *H.*

oryzae (ITCC 2537) both of which damage the crops such as maize and rice. Also they have observed that these compounds show no adverse phytotoxicity up to the concentration 10^{-3} M. Sen Sarma *et al.* [43] have described the biological activity of these compounds, with general formulae [$R_2Sn(OArCH{=}N{-}N{=}CSNH_2)$], where R=Me, n-Bu, Ph and Ar= $-C_6H_4$, $-C_6H_3$(5-Cl), $-C_6H_3$(5-Br), against four fungal pathogens (*Curvularia eragrostidi, Alterneria porri, Dreschlerea oryzae* and *Macrophomina phaseolina*) of four different crops (*Camellia sinensis, Guizotia abyssinica, Oryzae sativa* and *Solanum melongena*).

A wide range of organotin substitute show biocidal activity such as organotin compound with oxygen and nitrogen donor legends [44-47]. Even, the organotin complexes of sulphur containing legands have been found biological applications [48-49]. Rehman *et al.* [50] presented the synthesis and *in vitro* antifungal activity of some Schiff bases and their organotin(IV) complexes against plant pathogenic fungi and it was found that they pose excellent fungicidal activity. The application of multi criteria decision-making methods to the results of *in vitro* antifungal properties of oraganotin compounds of the type Ph_xSnX_z (x=2 or 3; X=$O_2CC_6H_4OH$, $O_2CC_6H_4OCOCH_3$, Cl or O_2CCH_3; z=1 or 2) and of free 2 hydroxybenzoic and 2-acetoxybenzoic acids against many fungi such as *Aspergillus niger* and *Aspergillus flavus* etc. was described by Gorwin *et al.* [51]. The [nBu_3Sn(N-phthaloylglycinnate) OH_2] and 3 other triorganotion derivatives of N-phthaloyl-protected amino acids were tested by Ng *et al.* [52] and the complexes were having a varying degree of inhibitory effect against several economically important plant pathogenic fungi like *Alternaria padwickii*, *Batryodiploddia theobromae*, *Colletotricum musae*, *Pestalodiopsis guepin* and *Phytophthora palmivora*. Triorganotin(IV) compounds appear to inhibit the mitochondrial function in Dutch elm diseases of American elm tree [53,54]. Eng *et al.* [55] observed that complexes of several $Ph_3Sn(IV)^+$ carboxylates and of some 1:1 addition compounds of Ph_3SnCl and 2,3-disubstituted thiazoliun 4-ones were effective against *Ceratocystis ulni,* a pathogen of Dutch elm diseases.

Additionally, $Ph_3Sn(IV)^+$ compounds of p-ethoxybenzoic acid and acetyl salicylic acid contain molecular units with distorted tetrahedral Sn center. These complexes have significant inhibitory activity against a range of fungi [56]. Kalsoom *et al.* [57] reported that a series of di- and tri-organotin complexes of 2-thionapthene had

moderate biological activities against various bacteria and fungi. The fungicidal activities of ArSn(IV) compound (p-Z $C_6H_4)_3SnX$ where X= $^-$OAC group, $^-$OH or ½ O, Z=F, Cl, CH_3, CH_3O, C_2H_5 or $[(CH_3)_3C]$ was reported by Whaef *et al.* [58]. They found that p-substitution reduces biocidal activity but p-CH_3O was completely ineffective. Based on these experiments, they proposed a model for the fungicidal action[58]. In view of the promising results, some organotin(IV) dithiocarbamates of the formula $RnSn(SCSNR_1R_2)_{4-n}$ (R = Ph or Bz; R_1 = R_2 = alkyl or aryl; n = 1 or 2) were synthesized as well and evaluated *in vitro* against five fungi [59]. Triphenyltin(IV) phenylthiocarbamate had the best overall antimicrobial activity. A series of organotin(IV) complexes of pipyridyl dithiocarbamates of the types R_2SnL_2, R_3SnL [60] and R_2SnLCl [61,62] also exhibited high activity compared to free ligand against bacteria and fungi. A large number of organotin(IV) complexes of compositions Ph_3SnL•bipy and Me_2SnLCl•bipy (L = anion of amino acid, e.g. tyrosine or phenylalanine) have been screened against a number of fungi and bacteria to assess their growth inhibition potential [63]. The organotin(IV) derivatives of the amino acids have been of interest as possible biocides [64–66]. Tri*cyclo*hexyltin(IV) alaninate has been found to be active as a fungicide and bactericide for seeds and plants [67]. Organotin(IV) complexes of amino acids [68–70] of the type R_3SnL and R_2SnL_2 (R = Me, Ph or n-Bu, L = anion of various amino acids) were found to be active against a wide spectrum of bacteria and fungi. Organotin(IV) complexes of extended systems derived from the condensation of 2-amino-5-(*o*-anisyl)-1,3,4-thiadiazole with salicylaldehyde, 2-hydroxynaphthaldehyde and 2-hydroxyacetophenone, were also screened *in vitro* against the same panel of bacteria and fungi [71,72]. The effect of fentin acetate against various fungi and bacteria was investigated. They are *Phytopthora palmivora* of black pepper [73] , *Pyrenopezziza brassicae* of brassicas [74], *Perenorporm destructor* of onion [75], basal stem rot diseases of cowpea [76]. The fentin hydroxide was also effective against various fungi e.g *Cercospora beticola* of sugerbeet [77] and *Stemphylium solani* of wheat [78] etc.

5.2.2 Economic importance of *B. sorokiniana*

The theoretical aspects including methodology of yield losses in general crops as well as for economically important commercially cultivated crops have been

reviewed [79-84]. The importance of yield losses in relation to host age is the most important particularly in designing strategy of management and decision making. In a conservative estimation, it has been reported that the yield losses due to foliar blights in wheat ranges from 20 to 100% [4-5] depending on genotypes and environmental factors (Fig. 5.2).

Fig. 5.2 Foliar blight diseases appeared in wheat plant on the field trial condition. (Inset: a close up view in the top right corner)

At Poza Rica, in Mexico which is the hot spot for screening the resistance to spot blotch diseases, 49-90% yield losses have been recorded by Duveiller *et al.* [85]. In the Mixteca region of Mexico, on-farm trials under severe natural infection by tan spot diseases indicated plots under zero tillage suffer loss in yield around 37% even with one spray of propiconazole [86]. In Argentina, tan spot is recognized as major leaf blight in wheat and severity level beyond 50% is a common feature with potential yield losses up to 20% [87]. In Paraguay, during 1972, losses due to the disease complex in the wet year reached as high as 70% [88,89]. In Pakistan, previously the spot blotch was considered to be of the minor importance [90]. However in 2000 during a survey of wheat fields in various districts of Punjab, foliar spots were observed in different frequencies [91]. Later *B. sorokiniana* was found the

predo[illegible]athogen of foliar spot in all wheat growing areas o[illegible]kistan [92]. Due to this a[illegible]ive pathogen, the yield loss was estimated at18-22 % in India [20] and 23.8% in Nepal [93].

In farmers' fields of Bangladesh, the average yield losses due to foliar blights were estimated to be 15% [94] whereas yield losses due to spot blotch were about 20% in Sonalika cultivar, whereas 14 and 8% losses have been reported in Akbar and Kanchan cultivar respectively [95]. Sowing time also dramatically increases yield loss. For an example, in Bangladesh, the late sowing of the two leaf rust susceptible cultivars Sonalika and Kanchan resulted in a yield loss of 71% and 41%, respectively [96]. A study conducted at Bangladesh into the effect of *B. sorokiniana* on wheat production revealed that upon the artificial inoculation of plants at the flag leaf stage reduced the number of grains per year head and 1000-grain weight by 7-100 % and 12-100 %, respectively compared with the control [97]. In Nepal, the yield losses have been registered around 27 % [98].

The losses caused by *A. triticina* and *Helminthosporium* spp. have been estimated separately in India. Flag leaf in almost all cereals has major impact on yield potential of the concerned crop. Chenulu and Singh [99] estimated losses due to *A. triticina* to the extent of 99% in a highly susceptible variety, Sonalika under artificial conditions of inoculations at the boot stage in pot experiment. Nema and Joshi [100] correlated reduction in grain weight to the number of lesions incited by *Helminthosporium sativum* and disease intensity per unit area of flag leaf in pot experiments. Prabhu and Singh [101] estimated the relative effects of *A. triticina* and *Helminthosporium sativum*, independently and in combination on yield and magnitude of losses .They observed that losses increased at the rates of 0.92, 0.52 and 0.36 per unit increase of the disease incited by *Helminthosporium sativam*, *A. triticina* and combined infection of both the pathogens respectively. Studies conducted at Rajendra Agricultural University, Pusa, India revealed that 20-25% loss in grain yield is inflicted due to blights in different wheat cultivars under normal years, which may be more in epiphytotic years [102].

The yield losses due to foliar blight at Faizabad, India during 1994-95 crop season, was estimated to be 20 and 22% in the wheat varieties UP 262 and HD 1633, respectively [103]. Yield loss assessment conducted at Cooch Behar, West Bengal

showed 42% yield loss in highly susceptible varieties in experimental plots and on farm studies up to 21% [104].

5.2.3 Symptoms of *B. sorokiniana* infection

The *B. sorokiniana* is an aggressive pathogen that causes spot blotch, root and crown rots, node cankers, ear head and seedling blight in wheat [105]. Lesions in leaves are small, chlorotic and oval shaped with dark centers. Lesions have reddish brown centers with yellow margins and tapered ends. They may reach several centimeters before coalescing and inducing the death of the leaf. If spikelets are affected, it can result in shriveled grain and black point, a dark staining of the embryo at the end of the seed [106].

5.2.4 Management of foliar blight

The management of foliar blight by different pathogens was the centre of interest for controlling the severity of the diseases. It becomes critical particularly under favourable environmental regime for fungal pathogens, as they are highly sporulating with shorter life cycle as well as easy dissemination. Generally multipronged strategy becomes essential and application of a single tactics results in obvious failure. Conventionally foliar spray has been a first choice to researchers, extension officers and to a practicing farmer. Integrated pest management (IPM) with various stages of development in different countries has been attempted, however, not yet accepted in larger scale among the growers' community. Researches relevant to foliar blight management can be considered and categorized as component for developing IPM.

5.2.4.1 Seed treatment

Seed treatment with different fungicide and various other substances including non-conventional chemical may provide variable protection against foliar blight incidence of wheat. Hait and Sinha, [107] reported that phytoalexin inducers like cupric chloride, ferric chloride at 10^{-3} M protected the wheat seedlings from foliar

blight infection. The other effective fungicides include captaf, mancozeb, thiram , pentachloronitrobenzene, praline and triademefon [108,109]. The foliar pathogen can be controlled with seed treating fungicides like guazatine and guazatine along with imazalil [110]. Pavlova *et al.* [111] indicated that seed treatment with some dual fungicides combination such as flutriafol and thiabendazole carboxin and thiram, difenoconazole and cyproconazole, tebuconazole and diniconazole provided protection against root rot caused by *Fusarium, Helminthosporium, Bipolaris* and *Rhizoctonia* spp. In 2002, Domanov [112] conducted field trials to test carbendazim along with carboxin which gave good control of root rots and increased yield of wheat. The efficacy of Raxil (tebuconazole) was studied on spring wheat cultivar as seed treatment with tebuconazole and found to control root rots caused by *B. sorokiniana* (*Cochliobolus sativus*) and *Alternaria* spp. [113]. Seed treatment with Vitavax 200 B and Bavistin increased seed germination by 43% and reduced seedling infection by *B. sorokiniana* in Nepal [114]. Seed and soil borne inoculum are the most important sources in the establishment of spot blotch on wheat. Seed treatment with Vitavax-200^{TM} was consistent in a 10% yield increase or higher, across Bangladesh [115]. In 2009, Malaker and Mian [116] reported the efficacy of seed treatment and foliar spray with fungicides in controlling black point caused mainly by *B. sorokiniana* and *A. alternata* incidence of wheat seeds was evaluated in the field. Two seed treating fungicides, namely Vitavax-200 and Homai-80WP were used @ 0.25% of dry seed weight and foliar spray with Tilt-250EC (0.05%) was applied in six different schedules.

The seed treatment of a newly developed fungicidal formulation, Vitavax 200 WS (carboxin + thiram in a ration of 1:1) @ 2.0, 2.5 and 3.0 g/kg gave good results in reducing seedling mortality, incidence of foliar diseases at multilocations of India including Uttar Banga Krishi Viswavidyalaya, West Bengal [117].

5.2.4.2 Chemical management

The use of fungicides for the control of foliar blight has been attempted in many countries including India with mixed success despite the harmful effect of fungicides; it had proved useful and economical in the control of tan spot and spot blotch [118].

In Brazil, Mehta [19] observed that foliar fungicides are an effective in controlling spot blotch. Non systemic and systemic foliar fungicides belonging to the dithiocarbamates (*namely*, mancozeb) and triazoles (*namely*, propiconazole, tebuconazole, flutriazol, procloraz, and triadimenol) and dicarboxymides (namely, iprodione) are known to be effective. The first spray should be applied soon after the onset of disease symptoms appear. In a non-endemic zone generally spraying may be done 45-55 day after sowing for the spring wheat cultivars [119]. Foliar applications especially with systemic fungicides such as tebuconazole, epoxiconazole, flutriafol, cyproconazole, flusilazole, epoxiconazole, and metaconazole, applied between heading and grain filing stages, have been proved to be cost effective. Under severe disease infestation, a second spray can result in a grain yield increase by 38-61% [120].

The fungicide, manzate reduced disease severity by 25%. There were no significant differences between two treatments reduced the average disease severity on both flag leaf (F) and (F-1) leaves by more than 75%. Only two sprays of tebuconazole (Folicur) caused a significantly larger reduction in disease severity (86%) [86].

In Argentina, Annone [121] observed positive response with foliar fungicides for reducing disease development under conditions of moderate to low inoculum pressure, but inconsistent results under high inoculum pressure. Among fungicides tested under field conditions, tebuconazole and propiconazole achieved the highest level of control, though results varied widely (30-80%).

In 2003, Tewari and Wako [122] reported that the mixture of tebuconazole and metacid applied as foliar spray at the boot stage of wheat crop effectively suppressed all the foliar diseases (brown and yellow rusts, powdery mildew and leaf blight) showing curative property with no phytotoxic effect on the plant. They also observed that Sencor and Propiconazole (Tilt) was incompatible and highly phytotoxic. They further noticed that the mixture of zinc sulphate and urea completely inhibited mycelial growth of *B. sorokiniana* and also inhibit 78% growth of *A. triticina*. Rashid *et al.* [123] reported propiconazole to be very effective against foliar blight of wheat (*B. sorokiniana*). The different fungicides like flusilazole, prochloraz, propiconazole and tebuconazole were effective against tan spot disease of wheat [124]. In 2008, Singh *et al.* [125] observed that the foliar sprays of Propiconazole @

0.1% beginning from the appearance of the disease and later at 15 days intervals thrice reduced the leaf blight incidence and increased grain yield at Karnal, Pantnagar, Faizabad, Dharwad and Cooch Behar in India. At Uttar Banga Krishi Viswavidyalya, the management of foliar blight has been attempted in an integrated approach and it was observed that seed treatment with carboxin and single spray with propiconazole (Tilt) at panicle initiation stage was effective in reducing the disease symptoms [22]. An experiment was undertaken to find out an effective integrated approach in controlling Bipolaris Leaf Blight (BpLB) as well as foot and root rot diseases of wheat under field condition. Sixteen treatments consisting of chemical fertilizer alone in combination with soil treatment (poultry refuse) and fungicide (Tilt 250EC) were considered for the management of Bipolaris leaf blight as well as foot and root rot diseases of wheat caused by *B. sorokiniana* and *Sclerotium rolfsii* or *Rhizoctonia solani* respectively. Considerable differences were observed among the treatments regarding the disease severity, incidence, disease control and grain yield. Disease severity increased both at lower and higher doses of N that is at '0' and '150' kg N/ha, respectively. Disease severity was reduced significantly through the use of recommended chemical fertilizers (N_{100} P_{26} K_{50} S_{20} B_1). Addition of poultry refuse (1.5 tons/ha) and Tilt 250EC (0.5 ml/L) with chemical fertilizers further reduced disease severity resulting the highest grain yield (4956 kg/ha). This yield was higher over the farmers practice, N_{100} P_{26} K_{50} S_{20} + poultry refuse + Tilt 250EC and N_{50} P_{13} K_{25} S_{10} B_1 + poultry refuse + Tilt 250EC, by 20%, 17% and 15% respectively [126]. The best chemical way to protect yield loss from spot blotch is to foliar spray a fungicide combined with seed treatment. From a number of years of experiments the average yield loss due to spot blotch was estimated at 15%. After conducting a series of experiments with varying rates of fungicides and from an economic and environmental viewpoint, a single spray of fungicide Tilt 250EC (125 a.i./ha)combined with seed treatment (Vitavax-200TM @ 3g/kg of seed) at the dose ml/L of water/20m^2, at 35-50 day after sowing (booting to heading) was found profitable for successful wheat production (Banu *et al.* Personal communication)

5.2.4.3 Induced resistance

Yield losses in wheat and barley leaf blight (*B. sorokiniana*) indicate the need to search for alternative strategies of disease control. Globally, one of the emerging

strategies is induced resistance. In the broadest sense, induced resistance means the control of causal agents by a prior activation of the plants own defense system. Defense was activated by necrotizing pathogen as well as by chemicals mimicking factors of the natural defense systems, such as salicylic acid [127,128]. Chemical induction of resistance to *B. sorokiniana* in barley by pre-treatment with inducers 2,6–dichloroisonicotinic acid (DCINA), benzo (1,2,3) thiadiazole-7-carbothioic acid-s-methylester (BTH) or jasmonates leads to reduction of the diseases in the range of 10-20% (Kumar and Ibeagha, personal communication). In 1986, Hait and Sinha [107] reported that seed treatment with heavy metal ions provides the protection to the wheat seedlings from *Helminthosporium* infection.

The biocontrol efficiency of *Epicoccum purpurascens, Gliocladium roseum,* three strains of *Bacillus subtilis,* and *Pseudomonas fluorescens*, isolated from the rhizosphere of wheat plants, was assessed in relation to seedling blight caused by *B. sorokiniana*. An *in vitro* study of the potential antagonist was performed using the dual culture technique and by 'sowing' wheat seeds pelleted with the saprophytes in plates with water agar along with the pathogen. *In vivo* assays were carried out in the greenhouse and in the field with pelleted seeds sown in artificially infested soil. Both the number of living plants and the number of plants with necrosis on the leaves and the base of the stems and roots were assessed 15 days after sowing. Under greenhouse conditions, *B. subtilis* and *G. roseum* reduced the level of infection of Buck Pucar and Trigomax 100 cultivars, respectively. In the field, biocontrol of the disease was not achieved [129].

5.2.5 Biochemical changes due to infection

The morphological, physiological and biochemical characterizations of *B. sorokiniana* have been the major aim of many studies [130-134]. However, knowledge about the genetic structure of this fungus is less available [135-137]. In general, plants respond in two different ways to pathogens. There is either no obvious interaction, or an interaction occurs and is, in the extreme cases, either incompatible (where the plant is resistant) or compatible (where the plant is susceptible). The biochemical events occurring in interactions between host or non-host plants with potential pathogens are basically similar but their timing of appearance and both the

intensities and patterns depend on their genomic as well as environmental conditions [138]. It has been established that different plant species, even varieties within a species may have fine differences in their biochemical make-up particularly in respect of phenolics, proteins and various other primary and secondary metabolites variation in respect of constitutive components as also in their enzyme components. They also differ in their responses to infection in most of these respects. Such biochemical differences in the host responses to inoculation with their potential pathogens have been studied for many host-pathogen combinations and the sum of all available data suggest that most, or all of them, are part of a typical resistant response of plants, one often determining over the others as the major defense mechanisms. Variations may occur more at the level of timing of induction, location and relative amounts, than in terms of all-or-none response.

Sixty-seven isolates of *B. sorokiniana* of barley, belonging to three groups (black, white and mixed) were studied to find an association of melanin with the spore production of the fungus. Conidiogenesis in black, white and mixed subpopulation of *B. sorokiniana* was positively correlated with melanin content/g of mycelium. Primary hyphae of black and mixed subpopulation differentiated into secondary hyphal structures which subsequently produced conidiophores and conidia. Primary hyphae could not differentiate into secondary hyphae and subsequently conidiophores and conidia in white subpopulation. A melanin containing mutant developed from white subpopulation regained its ability to differentiate into secondary hyphae, conidiophores and conidia. Results showed that melanization of mycelia *B. sorokiniana* mycelia is an important factor for conidia production [139].

5.2.5.1 Phenol

Since Newton and Anderson [140] suggested in their 'Phenol hypothesis' that resistance of wheat to rust fungus was due to the accumulation of phenol caused by the fungal entry and its subsequent inhibition of the parasite, there by imparting a large amount of information accumulated on the possible role of phenol in disease resistance. Later several reports suggested that rapid synthesis of phenolics following infection to be an important first line defense in plants [141].

The interaction of plants with the pathogens for post-infectional increase in phenol level has also been reported [141- 143]. Reddy *et al.* [144] reported post-infectional increase in phenol level in groundnut plants when infected with *Rhizoctonia solani.* The resistant groundnut varieties contain more phenols than susceptible ones and also responded to infection with *Cercospora* sp. with greater increase in phenol [145].

5.2.5.2 Ortho-dihydroxyphenol

Ortho-dihydroxyphenols like chlorogenic acid have often been implicated in disease resistance reactions. They are easily oxidized by polyphenoloxidases and the resulting quinones are highly reactive and toxic to pathogens by inhibitory to their enzymes, particularly chain splitting pectic enzymes [146].

5.2.5.3 Protein

Plants are known to undergo both quantitative and qualitative changes in their protein content upto infection. The synthesis of 'pathogenesis- related protein'(PR protein) is induced not only by infection with pathogens or treatment with pathogen derived elicitors but also by exposure of plant tissues or cultured plant cells to various inorganic or organic chemicals [147].

Synthesis of new proteins in resistant host varieties following infection with the pathogen has been reported for several plant species such as cucumber-*Colletotricum lagenarium* [148], tobacco- *Thielaviopsis basicola* [149] interactions. In tomato plants, resistant to *Cladosporium fulvum* some novel proteins appear soon after infection [150]. Working with brown spot of rice, Hait and Sinha [143] observed that the disappearance from the susceptible rice plants protected from *H .oryzae* by seed treatment with cysteine and sodium selenite, of three common proteins that they share with the pathogen and also the appearance at the same time of two new proteins in them having similar Rf values as two proteins present in resistant plants.

5.2.5.4 Polyphenoloxidase

Changes in host physiology following infection or induced resistance is often associated with an activation of oxidase activity and post-infectional rise in the level of such enzyme is a common phenomenon in diseased tissue, more so in an incompatible interaction. The activity of polyphenoloxidase would seem to be important as it can oxidize phenolics to quinones which may be more fungitoxic. The infected resistant tissue shows in many cases a higher oxidase activity than the infected susceptible tissue as also in healthy one. Such observations have led to the speculation that stimulated polyphenoloxidase activity possibly contributes to the resistance of plants against the pathogen, an idea though not fully accepted. Various observations on the role of polyphenoloxidase in host resistance are well documented in literature [151-153].

5.2.5.5 Peroxidase

Increased peroxidase activity in response to infection is a common phenomenon in different host-parasite interactions and the greater activity is generally linked with incompatible than with compatible interaction, i.e., linked with disease resistance in the host though contrary evidence is also available. Importance of peroxidase in phenylpropanoid metabolism as the terminal enzyme in lignin biosynthesis is important for containing the spread of the pathogen in host defense. The role of peroxidase in plant defense has been attributed to its ability to catalyse various types of oxidative reactions important in metabolism of the pathogen or of the host plant such as phenolics, toxins, hormones, etc. [151,152]. Heitefuss *et al.* [154] observed that peroxidase activity differed in resistant and susceptible cabbage varieties to *F. oxysporum* f. sp. *conglutinans* and resistant plants showed greater activity following infection. Greater post-infectional increases in peroxidase activity in the resistant variety has been reported in groundnut- *Puccinia arachidis* [155], groundnut-*Cercosporidium personatum* [145], rice-*Helminthosporium oryzae* [143], rice-*Pyricularis oryzae* [156,157]and wheat-*Erysiphe graminis* [158] interactions.

5.2.5.6 Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL) activity was strongly reduced in barley and wheat leaves after inoculation with highly or weakly aggressive isolates of *B. sorokiniana*. Aggressive isolates, however, generated much stronger early induction of PAL than less aggressive isolates. Prior inoculation of barley leaves with non-pathogenic strain provided partial protection against a subsequent challenge with *B. sorokiniana* after seven days. Induced plants showed unchanged PAL activity levels compared to those of non-induced plants after challenge inoculation. The results suggest that PAL plays a role in the active defenses of barley and wheat in response to pathogen attack, but apparently not in response to non-pathogens, wounding, or in plants expressing induced resistance [159]. Kervinen *et al.* [160] used gene-specific probes to assess the expression patterns of four different phenylalanine ammonia–lyase (PAL) genes in infected or elicitor-treated leaves and suspension cultured cells of barley. Genes corresponding to *hpa*12, *hpa*13, *hpa*14 and *hpa*16 were all induced by mercuric chloride and fungal infection by *B. sorokiniana* in barley leaves, but with considerable variation in their expression level and timing. Mycelial preparation of *B. sorokiniana* causes delayed induction of phenylalanine ammonia-lyase activity as compared to crude extract and purified glucan [161]. Paltonen and Karjalainen [162] suggested that second phase of PAL induction in wheat and barley is linked with resistance to *B. sorokiniana* infection.

5.2.5.7 Pathogenesis related (PR) proteins

Attempts have also been made to understand the molecular mechanism of this pathogen infection. Cross species hybridization was made using barley cDNA as probe against some rice transcript. The transcripts for the pathogenesis related proteins such as PR-1, PR-2, PR-3, PR-4, PR-5 and peroxidase were found to be up-regulated in response to *B. sorokiniana* in rice [163]. PR-protein transcripts accumulation was seen 12h after infection with *B. sorokiniana* as well as after exposure with UV light. These transcripts reached to maximum accumulation levels at 24 h and all declined thereafter with the exception of the *PR*-4 transcript in response to *B. sorokiniana*. Maximum accumulation of the peroxidase transcript occurred at 12 h in response to *B. sorokiniana* and UV light.

5.3 Scope and objective

Organotin chemistry is a subject of interest for years due to not only of its rich structural chemistry but also for its versatile applications. Author is interested to explore the biocidal activity, specially related to plant pathogenic organisms ofnewly synthesized as well as previously synthesized compounds in this laboratory. Diorganotin complexes of salicylaldehyde thiosemicarbazone or di- and triorganotin complexes of 2-marcapto isothiocyanate have contained O, N, S and N, S atom as donor. Organotin compounds of O, N, S donor ligands are well known for their biological activity [43-50]. Crops are vulnerable to the attack of various fungi and bacteria apart from insects and pests resulting in to the mass destruction of considerable amount of important crops annually. Therefore, it is needless to say that we need biologically active molecule to protect our crops. The synthesized compounds achieve effective control of foliar blight disease of wheat, which is the important limiting factor of wheat cultivation in North-East Indian plane zone. The mechanism of action of this compounds on host physiology in respect of phenolics pathogenesis-related protein, enzyme such as polyphenol oxydase, peroxidase and phenyl alanine ammonia lyase and other biochemical parameters commonly associated with diseases resistance is investigated. So the newly synthesized compounds may serve as alternative agrochemicals.

5.4 Materials and methods

5.4.1 General comments

The solvents used in biochemical reactions were of AR grade and were obtained from commercial sources (Merck, India). The solvents were dried using standard literature procedures. Double distilled water was used. Used reagents were received from commercial sources (HiMedia, India).

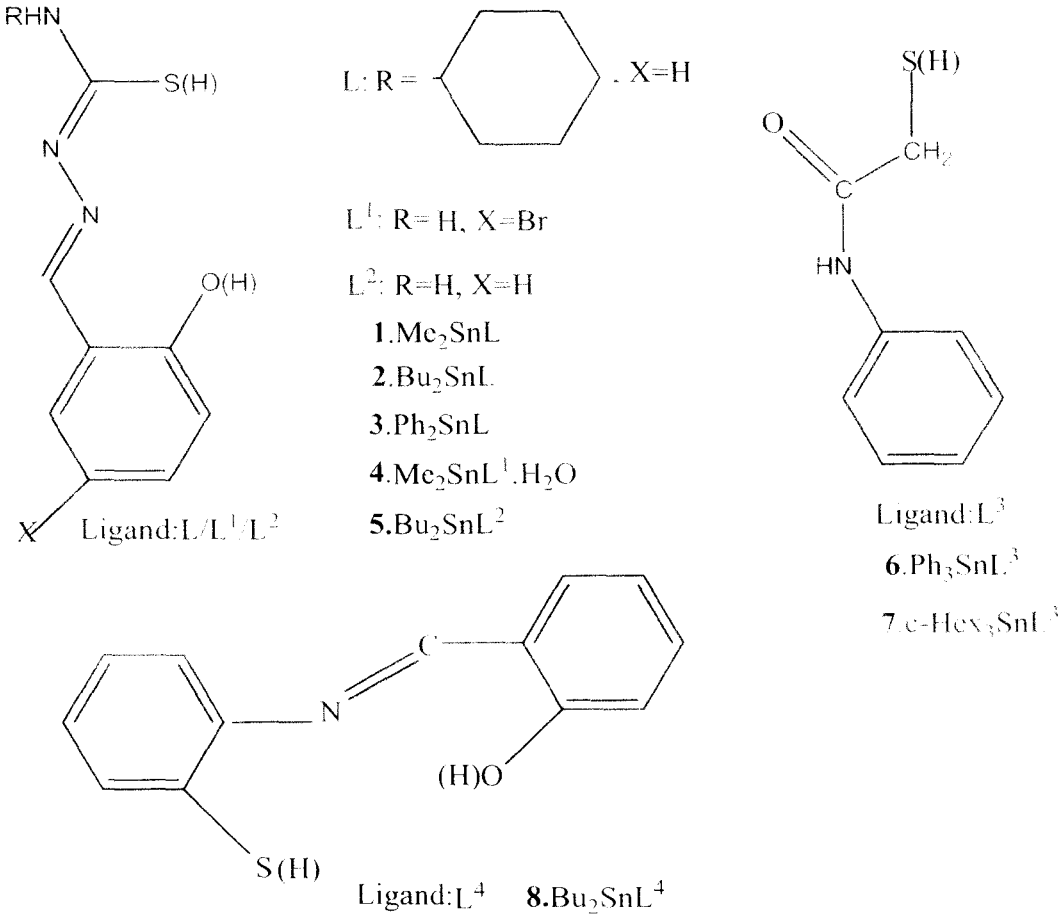
5.4.2 Measurements

The PAL enzyme activity was assayed by Parkin Elmer UV-Vis spectrophotometer in the optical density at 290 nm. The absorbance of the total phenol, ortho-dihydroxyphenol, protein, peroxidase activity, polyphenoloxidase

activity was measured by 722 Vis spectrophotometer, Jinghua instruments. Spore germination was counted by Lieca light microscope.

5.4.3 Synthesis and characterization of di and triorganotin(IV) complexes of Schiff bases

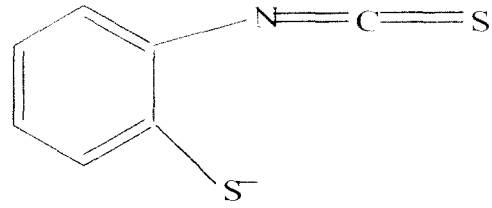
The synthesis and characterization of di and triorganotin(IV) of Schiff bases complexes (Set I) are described in Chapter 4 and in Sarkar *et al* [164]. In chapter 4, compound **8** is described as compound **4**. The formulae of the ligands and abbreviation of the complexes used for the present study are presented in Scheme 5.1.



Scheme 5.1.

5.4.3 Synthesis and characterization of di and triorganotin(IV) complexes of 2-marcapto isothiocyanate

The synthesis and characterization of di and triorganotin(IV) of 2-marcapto isothiocyanate complexes (Set II) are described in Chapter 3. The formulae of the ligands and abbreviation of the complexes used for the present study are presented in Scheme 5.2.



1. Me_3SnL; **2.** $n\text{-}Bu_3SnL$; **3.** Ph_3SnL; **4.** Bz_3SnL; **5.** $c\text{-}Hex_3SnL$
6. $Me_2Sn(L)_2$; **7.** $n\text{-}Bu_2Sn(L)_2$; **8.** $Ph_2Sn(L)_2$; **9.** $Bz_2Sn(L)_2$

Scheme 5.2

5.4.4 Location

The experiments were carried out at Research Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari Cooch Behar, West Bengal, situated between 25°57' N and 27°N latitude and 88°25' E longitude. The laboratory experiments were conducted at Department of Plant Pathology, Faculty of Agriculture, Uttar Banga Krishi Viswavidyalaya, Cooch Behar, West Bengal.

5.4.5 Weather

The experimental domain comes under terai agroclimatic zone of West Bengal. It is the northern aspect of West Bengal and is spread along the Bhutan Hills of Kalimpong and Karseong in northern side, Assam on its eastern border and Bihar on the west. This subtropical zone has a humid climate endowed with long rainy season starting from 1st week of May, continuing up to the end of September having low to heavy rainfall, ranges between 2100-3300 mm per year. Around 80% rainfall comes from the southwest monsoon during June-September when temperature varies between 24-33.2°C at the maximum and to 7-8°C at minimum with relative humidity 58% in March to 87% in July. Barring a period between December-February when

the winter sets in, this area as a whole remains warm and humid. This diverse climatic condition renders complicity to the agro ecological condition subjecting the region more prone to multiplication of pathogenic organisms.

5.4.6 Soil Characteristics

Soil of this zone is generally sandy loam to loam in texture, acidic in reaction, high in raw humus content, low in water retention capacity, low to medium in total nitrogen content with a low rate of nitrogen mineralization, low to medium in phosphorous status with high phosphorous fixation, low to medium in potash content and low in calcium and magnesium status. Micronutrients which are deficient in this zone are boron, molybdenum and zinc.

5.4.7 Media

All the glasswares were sterilized in hot air oven at 160°C for 30 min. The culture mediums were sterilized in an autoclave at 1.02 kg pressure/cm^2 for 15 min. The following medium was used for isolation, maintenance, sporulation characterization and mass multiplication of the fungi.

Wheat Dextrose Agar

Wheat leaves	100g
Dextrose	10g
Agar	7.5g
Distilled water	500 ml

Wheat leaves of 30-35 day old were first cut into pieces (2-3 cm) and boiled in 500ml of water till the colour of water becomes green and leaves become soften. Then it was filtered through cheesecloth. Required volume of water was added. Agar was dissolved in water to the required volume in warm condition and autoclaved at 15 lb pressure for 15 min.

Potato Dextrose Agar

Peeled Potato	250g
Glucose	20g
Agar	15g
Water	1000 ml
pH	6.0-6.5

Peeled potato was made into thin chips, boiled in 500 ml of distilled water till they were soft enough and extracted. The extract was filtered through cheese cloth. To the extract the weighed quantity of dextrose was added. Agar was melted in the other half of water and mixed in potato dextrose solution and the volume was made up to a litre. Finally it was autoclaved at 15 lb pressure for 15 min.

Oat Meal Agar

Oat Meal	15g
Agar	15g
Water	500 ml

Oat meal powder 15g was added in 500 ml of water. It was boiled for 15 min. volume was made upto 500 ml with water again. Finally the total solution was autoclaved at 15lb pressure for 15 min.

5.4.8 Planting material

Seeds of wheat were collected from Directorate of Wheat Research, ICAR Karnal, India and CYMMT (South Asia office, Kathmandu, Nepal). Seeds were air dried and stored in drier at 37°C. Wheat seeds were sown in sandy loam (field soil mixed with farmyard manure in 3:1 proportion) contained in pots (12-15 plant/ 25cm diameter pot). Prior of sowing, seeds were treated with 0.1% $HgCl_2$ for one min. to remove superficial contaminants, followed by several washing with sterile distilled water. During the summer season (April-June) and rainy season (July - September) the pot experiments were carried out in the environment controlled polyhouse.

5.4.9 Isolation of pathogens

Large number of blight infected wheat leaf sample were collected from different farm trials of Uttar Banga Krishi Viswavidyalaya Research farm. The infected leaf samples were washed with mercuric chloride (0.1%) solution and again rewashed with sterile distilled water. The leaf samples having typical blight symptoms were cut into small pieces (4 mm^2). The leaf bits were placed in slant containing wheat bran extract dextrose medium and incubated at 22°C for 7 days. After 7 days these isolates were transferred in different medium to observe their sporulation.

5.4.10 Fungal culture

5.4.10.1 Source

The *B. sorokiniana* were isolated from Research Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar. Cultures were first grown in wheat dextrose agar medium. Then the cultures were sporulated in Oat Meal Agar medium and then transferred to the Potato Dextrose Agar medium.

5.4.10.2 Completion of Koch's postulate

Wheat seeds were surface sterilized with 0.1% $HgCl_2$ solution for one minute, washed with sterile distilled water and sown in earthenware pots containing sandy loam soil. Seedlings (21days old) were inoculated with *B. sorokiniana*. Infected leaves were collected, washed, cut into small pieces, treated with $HgCl_2$ (0.1%) for one min., rewashed with sterile distilled water and transferred to OMA (Oat Meal Agar) slants. After 10 days, the isolated organism were examined, compared with the original stock culture of *B. sorokiniana* and its identity was confirmed.

5.4.10.3 Maintenance of stock

The fungi were grown on OMA slants and stored under different conditions (5°C and 20°C). Apart from weekly transfer for experimental work at a regular interval culture of *B. sorokiniana* was examined in order to test its pathogenicity

5.4.11 Definition of field experiments parameters

Following unit were used for field experiment

Days to Heading (DH): An entry was considered to have headed when 50% of the shoots have the entire spike out of the flag leaf. Days to heading was calculated from the day of planting.

Days to Maturity (DM): When at least 50% of the peduncles are physiologically mature.

Plant Height (PHT): Distance in centimeter from soil level to the tips of the spikes excluding the awns of randomly selected plants.

1000-grain weight: One thousand kernels taken randomly from harvested in each plot were weighted to obtain-grain weight.

Grain yield: In grams per plot, adjusted to 12% moisture level.

5.4.12 Disease Assessment

The disease was visually scored using the double digit scale (00-99) developed as a modification of Saari and Prescott's severity scale to assess wheat foliar disease [165,166] caused by *B. sorokiniana* was recorded in the whole plot. [Double digit (DD) system: For example, if the reading was 93, i.e. 9 represent height of the plants on which disease was developed and 3 represent % infected by the disease or 30% necrotic or chlorotic lesions appeared on the leaves]. The AUDPC (Area Under Disease Progress Curve) was calculated using the following formula given by Das *et al.* [167].

$$AUDPC = \sum_{i=1}^{n-1} [(x_i + x_i)/2] (t_{i+1} - t_i)$$

Where, x_i is the foliar blight severity on ith date, the t_i is the ith day and n is the number of scoring dates. The AUDPC measures the amount of disease as well as the rate of progress, and has no units.

5.4.13 Parameters of laboratory experiment

5.4.13.1 Detached leaf assay

Pads of absorbent cotton wool soaked with respective chemicals were laid inside 20 cm × 30 cm rectangular trays. Five excised pieces of leaves were parallely placed in each of the trays and cut ends were covered with soaked cotton pads in the form of wicks. 10 ml of *B. sorokiniana* spore suspension (10^6 spores / ml) was inoculated in each tray.

Under field conditions, the respective chemicals were sprayed thrice at 10 days intervals starting from 45 days after sowing.

5.4.13.2 Phenol

Extraction

Fresh healthy leaves from plants were collected, washed with distilled water and used to extract phenol. Around 2g of fresh tissue was crushed in a mortar with a pestle in 5 ml of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was saved. The residue was re-extracted with another 5 ml of 80% ethanol, centrifuged for 10 min and the supernatants were pooled. The ethanol fraction was then evaporated to dryness in vacuum at 40°C. The residue was then dissolved in 2 ml of distilled water.

Estimation

The total phenol content was estimated using Folin-Ciocalteau reagent [168]. Around 0.1 ml of extract was pipetted into a graduated test tube and the volume was making up to 3 ml with distilled water. To each test tube 0.5 ml of Folin-Ciocalteau Reagent (1N) was added. After 3 min, 2 ml of 20% Na_2CO_3 solution was pipetted into each tube. The tubes were shaken well and heated on a boiling water bath for 1 min and then cooled under running tap water. The absorbance of the resulting blue solution was measured at 650nm in spectrophotometer. For comparison a reagent blank was run without any phenol extract was added in it. Total phenol was determined in catechol equivalent after comparing with the standard curve prepared from pure catechol. Total phenol was expressed as mg/g fresh wt of tissue.

5.4.13.3 Ortho dihydroxy phenol (OD Phenol)

Extraction

Fresh healthy leaves from plants were collected, washed with distilled water and used to extract OD phenol. 2g of fresh tissue was crushed in a mortar with a pestle in 5 ml of 80% ethanol water mixture. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant was saved. The residue was re-extracted with another 5 ml of 80% ethanol, centrifuged for 10 min. and the supernatants were pooled. The ethanol fraction was then evaporated to dryness *in vacuum* at 40°C. The residue was then dissolved in 2 ml of distilled water.

Estimation

The OD phenol content was estimated following the methods of Mahadevan and Sridhar [168]. About 1ml of extract was pipetted out into a graduated test tube and the volume was made up to 1 ml 0.5(N) HCl. 1 ml of Arnow's reagent ($NaNO_2$:10g, Na_2MoO_4: 10g, distilled water 100 ml), 2 ml of 1N NaOH were added and mixed thoroughly in room temperature following which the total volume of the reaction mixture was made up to 10 ml by adding water. Optical density was recorded in a Spectrophotometer at 515 nm. A blank was prepared for comparison by adding 1 ml of alcohol instead of tissue extract with all other reagents. Standard curve was prepared with different concentrations of catechol. Results were expressed as mg/g fresh wt of tissue.

5.4.13.4 Protein

Extraction

Fresh healthy leaves from plants were collected, washed with distilled water and used to extract protein. Leaf tissue 0.5g was mixed with 0.05 M Sodium Phosphate buffer (pH 7.2) in mortar with pestle at 4°C with sea sand. The mixture was centrifuged at 4°C for 10 min at 15,000 rpm and the supernatant was used as crude protein and immediately stored at -20°C for further use.

Estimation

Soluble protein was estimated following the method of Lowry *et al.* [169]. Around 25 µl of protein sample was pipetted out and made up the volume with water

to 1.0 ml in the test tube. To the sample, 5.0 ml alkaline reagent (0.5 ml of 1% $CuSO_4$ and 0.5 ml of 2% sodium potassium tartarate dissolve in 50 ml of 2% Na_2CO_3 in 0.1 N NaOH) was added. This was incubated for 15 min. at room temperature and then 0.5 ml of Folin-Ciocalteau reagent (diluted 1: 1 with distilled water) was added and again incubated for 15 min for colour development following which optical density (O.D) was measured at 660 nm. Quality of protein was estimated from the standard curve made using bovine serum albumin (BSA) as standard.

5.4.13.5 Polyphenol oxidase (PPO)

Extraction

Fresh healthy leaves from plants were collected, washed with distilled water and used to extract PPO. Leaf tissue were cut into pieces of 1-2 cm and crushed in ice with 5 ml of pre-chilled phosphate buffer (pH 6.6) per g of tissue. The crushed material was centrifuged at 2,000 rpm of 4°C for 30 min. The supernatant was decanted and stored at 4°C

Estimation

For enzyme estimation, around 1.0 ml of enzyme extract, 1.5 ml of phosphate buffer (pH 6.0) and 0.5 ml. of substrate pyrogallol solution were mixed thoroughly by repeated inverting the cuvette and immediately the initial reading was taken at 495 nm. Further readings were taken in every 5 min interval. The blank reading was taken with 3 ml of phosphate buffer [168].

5.4.13.6 Peroxidase (PO)

Extraction

Fresh healthy leaves from plants were collected, washed with distilled water and used to extract peroxidase. Leaf tissue were cut into pieces of 1-2 cm and 0.2g taken in a pre-cooled mortar with phosphate buffer of pH 6 (5 ml/g leaf tissue), a pinch of neutral sand which was ground with pestle at 4°C. The homogenate was then centrifuged at 15,000 rpm for 15 min at 4°C and the supernatant was used as the source of enzyme.

Estimation

For measuring the activity, 3.0 ml of 0.05 M pyrogallol, 0.05 ml of enzyme extract was taken and mixed thoroughly. The tube was then inserted into colorimeter at 420 nm. After the colorimeter had been adjusted to show '0' optical density, 0.5 ml of 3% H_2O_2 was quickly added to the tube which was then inverted once immediately reinserted into the colorimeter. The change in optical density (O.D) between 30 and 150 sec at 420 nm was used to plot peroxidase activity [170]. A change in the absorption by 0.01 per min was accepted as a unit of activity. Results were expressed as unit of activity / g fresh tissue / min.

5.4.13. 7 Phenylalanine ammonia-lyase (PAL)

Extraction

To prepare the enzyme extract about 5 g of tissue (leaves) were homogenized in 1.5 ml of 0.05 M Tris-HCl buffer (*p*H 8.5) containing 1.4 mM 2-mercaptoethanol (100µl). The resulting slurry was filtered through two layers of cheese cloth. The filtrate was centrifuged at 15000 rpm for 15 min at 4°C. The resulting supernatant used as crude enzyme extract.

Estimation

The reaction mixture contained the following chemicals:

50 mM Tris-HCL (pH 8.8)	1 ml
20 mM l-phenyl alanine	0.5 ml
Enxyme extract	0.1 ml
H_2O	0.4 ml
Total	2 ml

The above mentioned reaction mixture was incubated for 60 min at 30°C. The reaction was stopped by the addition of 0.25 ml 2N HCL. The cinnamic acid formed by vigorous shaking was extracted in 2 ml toluene (This was done by vigorous shaking of the reaction mixture and toluene).The toluene layer was separated. One ml of the separated toluene layer was dried with a pinch of anhydrous sodium sulphate and taken in 1 ml cuvette. The specific activity of the enzyme was expressed as µ moles of cinnamic acid produced/min/mg of protein. The PAL enzyme activity was

assayed by measuring the appearance of *trans*-cinnamic acid in the optical density at 290 nm [171]. Standard curve was prepared with different concentrations of cinnamic acid for comparison.

5.4.13 .8 SDS-polyacrylamyde gel electrophoresis of total soluble protein

Preparation of slab gel

Stock solutions

For the preparation of gel, the following stock solutions were initially prepared as described by Laemmli [172].

(A) Acrylamide and N, N'- methylenebisacrylamide

Acrylamide	29 g
N, N'- methylene bisacrylamide	1 g
Distilled water	100 ml

Solution was filtered and pH adjusted to 7.

(B) Sodium dodecyl sulphate

SDS	10 g
Distilled water	100 ml

(Stored at room temperature)

(C) Lower gel buffer (1.5 M Tris)

Tris	18.18 g
Distilled water	100 ml

pH was adjusted to 8.8

(D) Upper gel buffer (0.5 M Tris)

Tris	6.06 g
Distilled water	100 ml

pH was adjusted 6.8

(E) Ammonium per sulphate (APS)

Ammonium per sulphate	0.1 g
Distilled water (freshly prepared each time)	1.0 ml

(F) Tris-glycine electrophoresis buffer

(25 mM Tris Base ; 250 mM glycine)

For 5 X Stock

Tris Base	15.1 g
Glycine	94 g

In 900 ml of distilled water, pH was adjusted to 8.3. Then 50 ml of 10% SDS was added and volume made upto 1000 ml.

(G) For 1 X SDS gel loading buffer :

50 mM Tris Cl (pH 6.8)

10 mM β-Mercaptoethanol

2% SDS

0.1% bromophenol blue

10% glycerol.

Slab gel preparation

For slab gel preparation, two glass plates (17 X 19cm) were washed with dehydrated alcohol and dried. Then 1 mm thick spacers were placed between the glass plates and the two edges along with two sides of glass plates were sealed with grease and gel sealing tape which were then kept in the gel casting unit. Resolving gel solution was prepared as follows:-

H_2O	11.9 ml
30% Acrylamide mix	10.0 ml
1.5 M Tris (pH-8.8)	7.5 ml
10% SDS	0.3 ml

10% APS	0.3 ml
TEMED	0.012 ml

The gel solution was cast very slowly and carefully up to a height of 12 cm by a syringe. The gel was over layered with water and kept for 2-3 h for polymerization. Then stacking gel solution was prepared as follows:

H_2O	6.8
30% Acrylamide mix	1.7 ml
1 M Tris (pH-6.8)	1.25
10% SDS	0.1 ml
10% APS	0.1 ml
TEMED	0.01 ml

After polymerization of resolving gel, overlay was decanted off and a 13 wall 1 mm thick comb was placed. Stacking gel solution was poured carefully up to a height of 4 cm over the resolving gel and overlayed with water. Finally the gel was kept for 30 min for polymerization.

Sample preparation

Sample was prepared by mixing the sample protein with 1 x SDS gel loading buffer (final volume 80µl). All the samples were floated in boiling water bath for 3 min. After cooling , upto 80µl of each sample was loaded in a predetermined order into the bottom of the wells with a micro liter syringe. Along with the samples, protein markers consisting of a mixture of six proteins ranging in molecular weight from 30 to 200 KD [Carbonic anhydrase – 29,000 , Albumin (egg) - 45,000 , Albumin (bovine) – 66,000 , Phosphorylase b - 97,400 , β - galactosidase – 166,000 ,and Myosin - 205,000 ,] was treated as the other samples and loaded in a separate well.

Electrophoresis

Electrophoresis was performed at 25 mA for a period of 3 h until the dye front reached the bottom of the gel.

Fixing and Staining

For fixing the fixer solution was prepared as follows :

Glacial Acetic Acid	10 ml
Methanol	20 ml
Distilled water	70 ml

The entire gel was removed from the glass plates and then the stacking portion was cut off from the resolving gel. After that gel was soaked for 2 h in the fixer for fixing.

The staining solution was prepared as follows:

Coomassie Brilliant Blue R250	0.25 g
Methanol	45 ml
Distilled water	45 ml
Acetic Acid	10 ml

At first, gel were stained by staining solution for 2-3 h and finally soaked with destaining solution (Methanol:Distilled water: Acetic acid :: 4.5: 4.5:1) until the background become clear.

5.5 Result and Discussion

To evaluate the efficacy of different organotin compounds to be tested against foliar blight disease of wheat were initially screened at a range of three concentrations (25, 50 and 100 ppm) each for their possible fungitoxic effect on the spore germination and radial growth of *B. sorokiniana*. The compounds were categorized as Set I comprising of eight chemicals and Set II comprising of nine chemicals. The fungicide Propiconazole (Tilt) was kept as standard/check.

5.5.1 Set I:

Table 5.1: Effect of different organotin compounds on spore germination and growth of *B. sorokiniana*

Compound	Spore Germination %			Radial Growth of *B. sorokiniana* (cm)		
	Con. (ppm)			Con. (ppm)		
	25	50	100	25	50	100
1.	9.62	2.38	0	1.5	Nil	Nil
2.	4.44	4.14	0	Nil	Nil	Nil
3.	3.56	1.21	0	Nil	Nil	Nil
4.	10.73	3.55	1.17	1.2	Nil	Nil
5.	4.80	2.45	0	Nil	Nil	Nil
6.	8.14	4.74	3.51	1.1	Nil	Nil
7.	8.41	4.55	3.03	1.1	Nil	Nil
8.	5.12	4.44	0	Nil	Nil	Nil
Propiconazole (0.15 %)	12.36	-	-	-	1.6	-
Control (Water)	85.91	-	-	-	7.5	-
CD (P=0.05)	0.213	0.182	0.162	-	-	-

5.5.1.1 Effect of organotin compounds on spore germination and growth

It appears from Table 5.1 that all the compounds significantly inhibited the spore germination even at 25 ppm and the inhibition was more pronounced with the increase in concentration (Fig. 5.3 and Fig. 5.4). At 25 ppm compounds **2**, **3**, **5** and **8** exhibited spore germination in the range 3-5% whereas 85 % was recorded in case of water control. These compounds were more effective than propiconazole at the recommended dose of 0.15 %. The radial growth of *B.sorokiniana* was almost completely inhibited in all the compounds tested indicating that the newly synthesized organotin compounds are highly effective against *B. sorokiniana*.

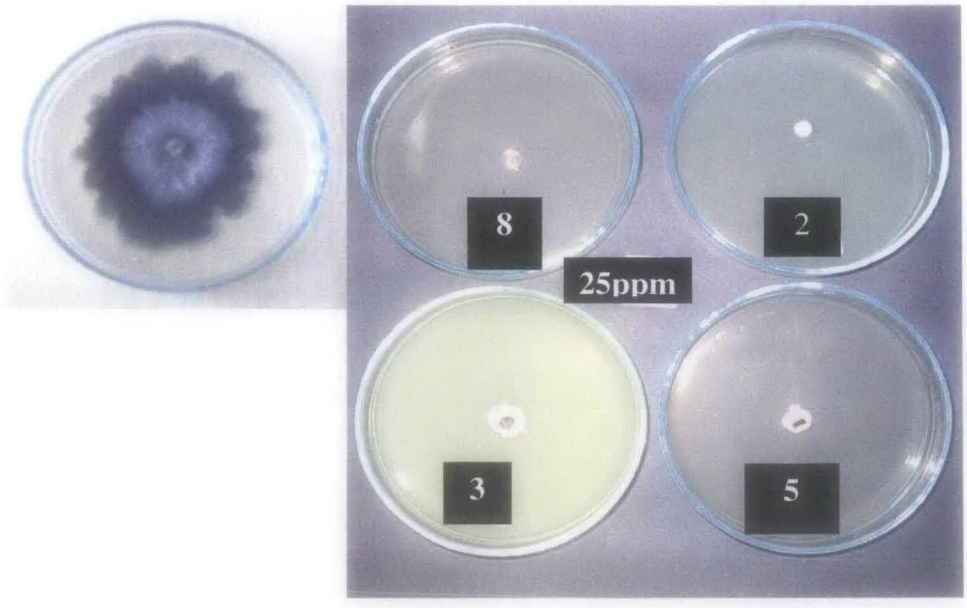


Fig. 5.3 Effects of test chemicals at 25 ppm on spore germination and growth of *B. sorokiniana* (control and treated).

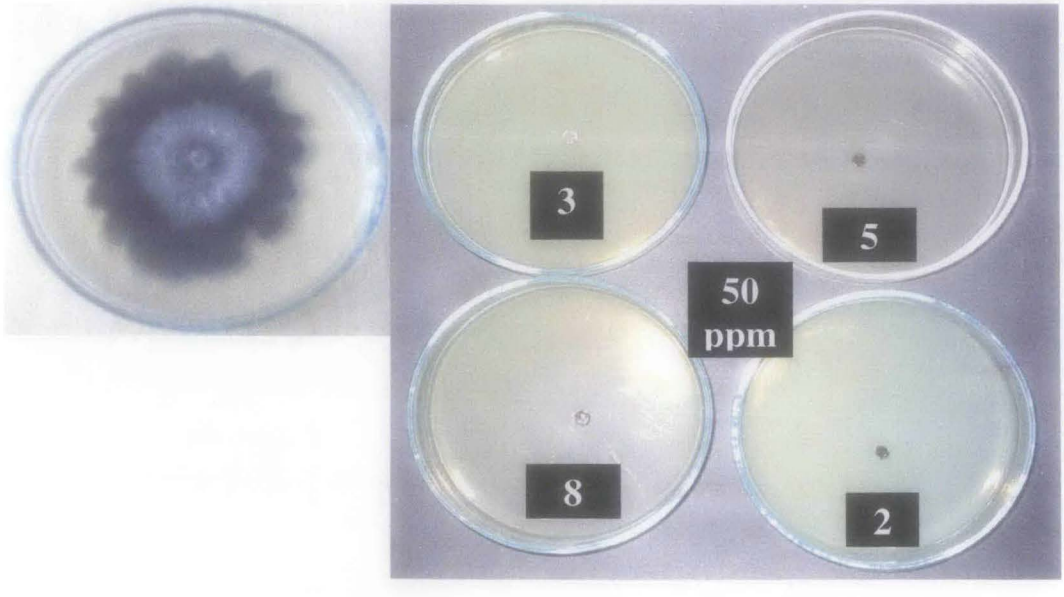


Fig. 5.4 Effects of test chemicals at 50 ppm on spore germination and growth of *B. sorokiniana* (control and treated).

5.5.1.2 Effect of organotin compounds on symptom expression and yield attributes in wheat plants

Eight organotin compounds have shown promise in the inhibition of spore germination of *B. sorokiniana* were further tested in field trials as well as detached wheat leaves by the methods described earlier for their effect on symptom expression and yield of wheat. The result is summarized in Table 5.2.

It appeared that susceptible plants in all the treatments showed very significant (P=0.05) differences in symptoms with the untreated plants both with excised wheat leaves and natural conditions as reflected by the reduced lesion area and AUDPC. As early as 72 h of inoculation, the lesion were fairly well developed in the untreated plants, those in different treatments showed only mild symptoms. Subsequently, symptom developments were distinctly higher in control, but in the treated plants 71% to 81% less symptoms than in the untreated plants were recorded. Among the different organotin compounds, **2**, **3**, **5** and **8** (Fig. 5.5) were more effective in reducing the disease symptoms. As recorded the average kernel weight (AKW), most of the treatments had little or no effect. The yield was also higher in the treated plants than in the untreated plants. The compounds, the most effective in reducing the disease symptom, also stimulated maximum yield of 46.7 Q/ha as that of the control which recorded 35.1 Q/ha (Fig. 5.6).

Fig. 5.5 A view of reduced disease symptoms compared to the control on excised wheat leaves by application of compounds **2**,**3**,**5** and **8**.

Healthy plants **Diseased plants**

Fig. 5.6 A view of field trial for organotin treated healthy and control (untreated) diseased plant.

Table 5.2: Efficacy of different organotin compounds on excised wheat leaves (cv. Sonalika) as well as leaves under field condition challenged with against *B. sorokiniana*.

Compound	Con. (ppm)	Average lesion area in(cm)		Area under diseases progress curve	1000 kernel wt. (g)	Projected yield Q/ha
		After 72 h of inoculation	After 96 h of inoculation			
1.	50	0.80	0.92	191.35	37.9	37.407
2.	50	0.82	0.90	188.85	37.8	44.458
3.	50	0.70	0.75	145.54	38.5	46.777
4.	50	0.85	1.25	188.85	37.8	40.747
5	50	0.82	0.84	160.53	38.4	44.444
6	50	0.84	1.12	302.46	37.2	36.155
7.	50	0.85	1.21	332.71	37.4	37.252
8.	50	0.84	0.95	203.70	37.5	43.505
Propiconazole	0.15%	1.70	1.92	256.09	37.3	36.574
Control (water)	-	3.3	4.25	622.20	37.2	35.111
C.D.(P=0.05)	-	0.197	0.131	-	-	-

5.5.1.3 Studies on the biochemical changes associated with resistance in wheat plants

It has been observed that different organotin compounds cause substantial protection against *B. sarokiniana* infection that continued to active over a period. Such effect is presumed to be mediated through some sort of conditioning of host tissue leading to activated dynamic defense and this may be associated with significant alterations in host metabolism in response to treatment as well as to infection. This possibility was investigated in a series of biochemical studies aimed at determining the nature of changes that occurred in susceptible wheat plants cv Sonalika. The areas of investigations include phenolics, proteins, polyphenol oxidase activity (PPO), peroxidase activity (PO), phenylalanine ammonia lyase activity (PAL), in healthy and infected tissues of cultivar Sonalika as influenced by organotin compounds.

Table 5.3: Effect of organotin compounds on phenol and OD-phenol contents in healthy and *B. sarokiniana* infected wheat leaves.

Compound	Phenol content (mg/g fresh tissue)						OD-phenol content (mg/g fresh tissue)					
	1st Day		3rd Day		7th Day		1st Day		3rd Day		7th Day	
	H	I	H	I	H	I	H	I	H	I	H	I
Water (Control)	4.43	4.48	5.14	4.51	4.81	3.31	0.223	0.228	0.224	0.221	0.224	0.187
Propiconazole	4.42	4.46	4.73	4.83	4.63	4.82	0.224	0.234	0.227	0.229	0.230	0.218
2.	4.90	5.05	4.79	5.15	4.41	5.10	0.223	0.236	0.227	0.240	0.229	0.235
3.	4.05	5.25	4.52	6.98	4.79	4.98	0.226	0.242	0.231	0.241	0.231	0.238
5.	4.45	5.13	4.18	5.96	4.45	5.67	0.224	0.233	0.230	0.236	0.230	0.235
8.	4.02	4.71	4.39	4.90	4.45	5.04	0.226	0.236	0.290	0.234	0.232	0.233
C.D. (P=0.05)	0.277		0.211		0.373		0.0094		0.0100		0.012	
H=Healthy, I= Inoculated												

5.5.1.3.1 Total phenols

Table 5.3 shows that inoculation resulted in a mild increase (5%) in phenol level in untreated leaves at 24h of inoculation but this effect rapidly decreased and infection resulted in small to perceptible fall (12-31%) in phenol level during the next 7 days. On the other hand, wheat leaves in different treatment recorded considerable increases in phenol level following inoculation at all three stages, 3-29% after 24 h, 11-52% after 72 h and 4-27% after 7 days. The final post-infection level of phenol in treated leaves was mostly significantly higher than that in the untreated plants. The wheat plants treated with propiconazole (Tilt) had moderate increases in phenol level at all stages of sampling but still fell slightly short of the levels in comparable organotin treated leaves.

5.5.5.3.2 Ortho-dihydroxy phenols

Treatment with organotin compounds either had no effect on the ortho-dihydroxyphenol level or caused only marginal to small increases in susceptible leaves (Table 5.3). In untreated susceptible leaves inoculation with *B. sorokiniana* resulted in a small increase (2%) in OD-phenol content, when sampled after 24 h, but at the later stages, between 3-7 day after inoculation, infected plants recorded moderate decreases (2-19%), the decline increasing with time. In different organotin compounds treatments, infection resulted in 4% to 7% increases in OD-phenol level within 24 h but the response gradually weakened with time. At all stages of sampling their post-infection levels were significantly higher than that in untreated plants. Among the different treatments, the different OD-phenol content is not statistically significant.

Table 5.4: Protein contents in wheat leave infected by *B. sorokiniana*

Compound	Protein Content (mg/g fresh tissue)					
	1st Day		3rd Day		7th Day	
	H	I	H	I	H	I
Water (control)	21.95	19.65	22.40	15.85	21.35	12.45
Propiconazole	21.65	25.90	21.95	21.15	23.60	17.90
2.	22.55	25.45	24.25	22.40	23.60	18.65
3.	22.65	25.25	23.45	23.35	23.95	18.60
5.	23.35	24.40	23.10	21.00	23.20	18.95
8.	22.05	24.60	23.90	21.75	22.60	19.75
C.D. (P=0.05)	3.251		4.082		2.675	
H= Healthy , I= Inoculated						

5.5.1.3.3 Total protein

Results in Table 5.4 show that susceptible untreated plants responded to inoculation initially with a mild decrease (11%) in protein content but at the later stages this effect further declined and recorded 24% lower after 3 day and 41% lower than the normal level after 7 day of inoculation. Susceptible plants in different organotin compounds initially respond to inoculation with a very mild increase (4-12%) in protein content, but the stimulatory effect also weakened in them with time and practically disappeared within 3 day of inoculation and after 7 day, the protein content was reduced to 18-20% lower than the normal level. The treated plants always had higher post-infection levels of total protein as compared to the comparable untreated plants, the quantum of differences varying between 24 % and 31% after 24 h as well as 32% and 47% after 3 day and at the later stage of infection the differences became more pronounced recording 49-58% higher than untreated plants. The plants treated with propiconazole had similar protein content with organotin treated plants at all stages of sampling.

Preliminary studies on the extraction of protein through gel electrophoresis show the appearance of an additional band indicating the formation of lower molecular weight pathogen related protein.

Table 5.5: Activity of peroxidase and polyphenoloxidase enzymes on wheat leaves infected by *B. sorokiniana*

Compound	Peroxidase (unit of activity/g fresh tissue/min)						Polyphenoloxidase (unit of activity/g fresh tissue/min)					
	1st Day		3rd Day		7th Day		1st Day		3rd Day		7th Day	
	H	I	H	I	H	I	H	I	H	I	H	I
Water (control)	70.5	90.0	69.0	87.5	72.0	83.5	0.080	0.100	0.085	0.060	0.080	0.040
Propiconazole	70.0	89.5	71.0	94.5	70.5	86.0	0.085	0.110	0.095	0.850	0.075	0.065
2.	70.5	94.5	68.0	100.5	71.0	93.0	0.080	0.120	0.090	0.115	0.095	0.105
3.	70.0	93.5	72.0	100.0	69.5	91.0	0.085	0.120	0.085	0.120	0.090	0.105
5.	71.0	91.5	70.5	101.0	72.0	93.5	0.080	0.120	0.085	0.110	0.085	0.105
8.	72.0	93.5	71.0	99.5	71.0	91.0	0.085	0.115	0.080	0.120	0.085	0.115
C.D. (P= 0.05)	7.51		9.08		8.30		0.0292		0.0299		0.0293	

H = Healthy, I = inoculated

5.5.1.3.4 Peroxidase activity

It will be seen from Table 5.5 that the untreated susceptible plants responded to inoculation with considerable increases in (26-28%) enzyme activity between 1 and 3 days after inoculation, but during the next 4 days, the stimulated activity strongly declined to almost normal. The treated plants, responded to inoculation with pronounced increases, 30% to 33% after 24h and 43% to 47% after 3 days. Though this treatment induced effect declined with time, still after 7 days i.e. at the late stage of infection, 29% to 31% higher levels could be noticed. All the treatments recorded significantly higher post-infection levels of peroxidase activity. Though maximum effect of treatments mostly occurred within 3 days of inoculation, generally the peak period for pathogenic activity still significant effects persisted even at the late stage of infection.

5.5.1.3.5 Polyphenoloxidase activity

Treated susceptible plants recorded very mild increases in the level of polyphenoloxidase activity than the untreated plants at different stages of sampling (Table 5.5). Following inoculation, polyphenoloxidase activity appreciably (by 25%)

increased in untreated susceptible leaves after 24h, however, this effect sharply declined during the 7 days, to much lower values than normal level. At every stage of sampling, susceptible plants in different treatments responded to inoculation with greater increases in enzyme activity, as much as 22% to 50% after 24h, 29% to 41% after 3 days and 10% to 35% after 7 days. Their post-infection levels were also much higher than in the untreated plants.

5.5.2 Set II

The nine organotin chemicals (**1-9**), screened for their possible effect in controlling foliar blight disease in wheat, were also tested for their effect *in vitro* on spore germination and radial growth of the pathogen, *B. sorokiniana*. Three concentration of each chemicals were tested for such assay in germination of spore kept in grove slide.

It appears that all nine chemicals were highly toxic to the pathogen at all three concentrations tested i.e. 25, 50 and 100 ppm (Table 5.1). Among the nine chemicals, compound **2** and **3** significantly inhibited the spore germination and radial growth of *B. sorokiniana* at 50 ppm (Fig. 5.7).

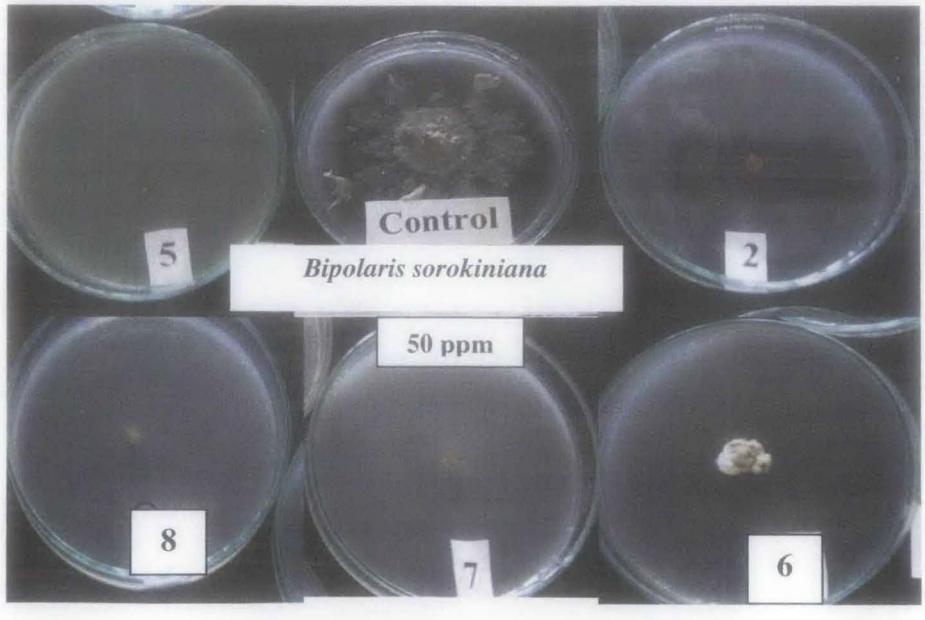


Fig. 5.7 Effects of test chemicals at 50 ppm on spore germination and growth of *B. sorokiniana* (control and treated).

5.5.2.1 Effect of chemicals on *B. sorokiniana* in wheat

As a follow up of the results just descried in Table 5.6, all the nine chemicals having significant inhibitory effects on spore germination and its growth were further tested for their possible effect on disease symptom inhibition on excised wheat leaves. Here only 50 ppm concentration of each chemical was selected. The results are shown in Table 5.7.

Susceptible plants in all the treatments showed very significant difference in symptoms with untreated plants as early as 72 h of inoculation (Table 5.2). Subsequently, symptom development was distinctly slower in the treated plants, so that after 96 h of inoculation these had very large difference in symptoms with control plants. Different treatments reduced disease symptoms by 54% to 83%. Best results were achieved with compounds **2** and **3**, even much better their standard fungicide, propiconazole.

Table 5.6: Effect of the test chemicals on spore germination and growth of *B. sorokiniana*

Compound	Spore germination(%)			Radial growth of *B. sorokiniana* (cm)		
	Con. (ppm)			Con. (ppm)		
	25	50	100	25	50	100
1.	4.44	2.38	0	Nil	Nil	Nil
2.	3.78	1.49	0	Nil	Nil	Nil
3.	3.21	1.21	0	Nil	Nil	Nil
4.	4.8	2.91	0.96	Nil	Nil	Nil
5.	3.56	2.45	0	Nil	Nil	Nil
6.	9.62	4.44	0.91	1.5	Negligible	Nil
7.	4.12	2.72	0	Nil	Nil	Nil
8.	4.82	2.72	0	Nil	Nil	Nil
9.	8.14	5.32	1.20	Negligible	Negligible	Nil
Propiconazole (0.15%)	12.06	-	-	1.6	-	-
Control (Water)	87.81	-	-	7.5	-	-
C.D.(P=0.05)	0.285	0.364	0.226	-	-	-

Table 5.7 Efficacy of organotin compounds on excised wheat leaves (cv. Sonalika) infected by *B. sorokiniana*.

Compound	Con. (ppm)	Av. lesion area (cm)	
		After 72 h of inoculation	After 96 h of inoculation
1	50	0.74	0.79
2.	50	0.71	0.75
3.	50	0.67	0.70
4.	50	0.85	0.90
5.	50	0.79	0.82
6.	50	0.90	1.35
7.	50	0.75	0.81
8.	50	0.80	0.93
9.	50	0.85	1.21
Propiconazole	0.15 %	1.70	1.92
Control (Water)	-	3.3	4.25
C.D.(P=0.05)		0.1229	0.0823

5.5.2.2 Biochemical changes associated with resistance in the wheat plant (cv Sonalika) after infection

Results of *in vitro* and *in vivo* experiment on the effect of organotin chemicals on foliar blight infection of wheat plants appeared to be promising. So it became of some interest to investigate if there is any correlation between post-infection biochemical changes in the treated plants and the resistance induced in them. It was decided in this connection to investigate host responses in respect of total phenol, ortho-dihydroxyphenol and protein content and polyphenoloxodase and peroxidase activities, parameters that have often been found to be associated with expression of disease resistance in plants. For these purpose leaf materials were collected from diseased plants in the control plants and those with 9 compounds with standard check, propiconazole after 24h, 72h and 7 days of inoculation. Results were described in below

Table 5.8 Effect of test organotin compounds on phenol and OD-phenol contents in wheat leaves (cv. Sonalika) infected by *B. sorokiniana*

Compound	Phenol content (mg/ g fresh tissue)			OD-phenol content(mg/g fresh tissue)		
	1st Day	3rd Day	7th Day	1st Day	3rd Day	7th Day
1.	5.98	6.12	5.04	0.210	0.221	0.167
2.	5.25	5.70	4.79	0.239	0.246	0.155
3.	6.09	6.22	5.41	0.278	0.289	0.187
4.	5.09	5.54	3.67	0.232	0.243	0.142
5.	5.72	5.80	4.25	0.222	0.228	0.178
6.	4.97	4.29	3.39	0.192	0.198	0.111
7.	4.89	5.02	4.31	0.240	0.248	0.172
8.	4.55	4.89	4.33	0.203	0.212	0.142
9.	4.87	4.99	3.37	0.198	0.207	0.131
Propiconazole	4.42	4.52	3.34	0.183	0.196	0.112
Control (Water)	4.33	4.02	2.78	0.177	0.173	0.040
C.D.(P=0.05)	0.096	0.145	0.127	0.013	0.009	0.009

5.5.2.2.1 Total phenol

It seen in Table 5.8 that infected susceptible wheat plants in all the treatments recorded higher (5% to 40%) total phenol levels as compared to the untreated plants after 24 hr. of inoculation. After 3 days of inoculation, the phenol level further increased in the treated plants but at the later stage of infection, this effect somewhat declined. The final post-infection levels in different treatments were 6% to 59% higher after 3 days and 32% to 44% higher after 7 days of inoculation as compared to the control plants. Plants treated with compound **3** that provided maximum protection also recorded maximum increase in the phenol level followed closely by compound **1** and **2**.

5.5.2.2.2 Ortho-dihydroxyphenol

The trend for Ortho-dihydroxyphenol was nearly similar to that for total phenol. All the treatments lead to appreciable increase, 8% to 57% after 24 h, 14% to 67% after 3 days and 177% to 367% after 7 days of inoculation. The correlation between the increase in OD phenol content and resistance was good but no absolute.

Table 5.9 Effect of different organotin compounds on peroxidase and polyphenol oxidase activity in *B. sorokiniana* infected wheat leaves (cv. Sonalika)

Compound	Peroxidase activity (unit of activity/g fresh tissue /min)			Polyphenoloxidase activity (unit of activity/g fresh tissue /min)		
	1st Day	3rd Day	7th Day	1st Day	3rd Day	7th Day
1.	73.7	74.0	71.8	0.94	1.00	0.90
2.	73.4	74.0	72.2	1.02	1.05	0.96
3.	76.0	80.3	74.0	0.98	1.1	0.97
4.	72.5	73.0	71.6	0.95	0.99	0.90
5.	77.5	79.4	74.0	1.00	1.01	0.92
6.	71.1	72.0	69.0	0.89	0.88	0.82
7.	75.0	77.4	72.8	1.02	1.04	0.92
8.	75.0	77.2	72.9	0.96	1.03	0.90
9.	73.6	74.0	70.0	0.93	0.95	0.85
Propiconazole	64.2	68.2	62.2	0.90	0.94	0.82
Control (Water)	72.4	62.2	50.0	0.88	0.70	0.55
C.D.(P=0.05)	1.251	0.959	1.189	0.119	0.138	0.102

5.5.2.2.3 Peroxidase activity

Table 5.9 indicates that plants in all the treatments recorded moderately higher peroxidase than untreated plants after 24 h and after 3 and 7 days were quite high after 3 and 7 days of inoculation. Though all the treatments recorded significantly higher post infection level of peroxidase activity, the more effective compounds like **2**, **3** and **5** recorded higher level of enzyme activity than less effective compounds, though the difference are not always significant (P=0.05).

5.5.2.2.4 Polyphenoloxidase activity

Following infection, the polyphenoloxidase activity was sharply declined with age in the untreated plants whereas the plants in all the treatments respond to inoculation with greater increase in enzyme activity. The final post-infection level varying between 2% to 15% after 24 h 25% to 57% after 3 days and 49% to 74% after 7 days. The maximum increase in the enzyme activity in all respect were recorded with the compound **3**, the most effective compound. Plants treated with propiconazole had intermediate effect in enzyme activity.

Table 5.10 Effect of organotin compounds on protein contents in *B. sorokiniana* infected wheat leaves cv. Sonalika.

Compound	Protein content (mg/ g fresh tissue)		
	1st Day	3rd Day	7th Day
1.	20.32	17.96	12.57
2.	25.37	20.62	13.48
3.	25.54	20.66	13.57
4.	23.49	20.21	12.92
5.	21.31	18.71	12.91
6.	18.63	15.24	11.34
7.	20.66	17.95	12.04
8.	24.71	20.32	13.57
9.	22.01	18.63	12.73
Propiconazole	20.17	17.12	11.87
Control (Water)	16.94	12.57	5.39
C.D. (P=0.05)	0.148	0.124	0.146

5.5.2.2.5 Total protein

All nine treatments recorded higher post-infection protein level than the untreated plants (Table 5.10). All the initial stages of inoculation the protein content were higher in the treated plants but with the time the level gradually decreased in these plants. The final post-inflectional levels were appreciably higher than that of

untreated plants and with time this difference became more pronounced after 7 days of inoculation.

5.5.3 Phenylalanine ammonia lyase activity

It has been clearly established that in the early experiments the degree of resistance was correlated with an increased biosynthesis of phenolics and stimulated oxidase activity at and around the site of host-pathogen interaction. It is well known that phenyl alanine ammonia lyase (PAL) activity is the first enzyme of the phenyl propanoid pathway and considered as the key enzyme in the regulation of the flux of the phenylpropanoid compounds such as lignin and their derivatives [173] and also appeared to be associated with hypersensitive reaction [174]. The PAL activity was measured in ten compounds that provided strong resistance against foliar blight pathogen excluding propiconazole after inoculation with *B.sorokiniana* and the results are presented in Table- 5.11

The PAL activity in the organotin compounds treated plants were always higher than untreated plants. A strong correlation were observed between resistance induced by the compounds and PAL activity mild variation.

Table 5.11 Effect of different organotin compounds on phenylalanine ammonia lyase activity in *B. sorokiniana* infected wheat leaves (cv. Sonalika)

Compound		PAL activity (µg cinnamic acid released/g/min)	
		24 h after inoculation	72 h after Inoculation
Set I:	**2.**	372.8	352.2
	3.	365.5	344.7
	5.	361.5	329.1
	8.	370.1	347.1
Set II:	**1.**	353.0	334.3
	2.	365.8	340.1
	3.	346.0	338.4
	5.	357.1	340.0
	7.	354.9	333.5
	9.	349.7	351.7
Propiconazole		329.9	315.2
Water (Control)		315.7	288.6

5.5.4 Effect of organotin compounds on seedling growth of wheat

The newly synthesized organotin compounds were further tested for their phytotoxic effect if any, on seed and its growth. For this purpose wheat seeds of Sonalika cultivar were tested with organotin compounds in set I and set II at 50 ppm concentration for 6 h and placed in the petridishes covered with moist blotter separately. The seed germination percentage was recovered after 72 h of treatments and root as well as shoot length were recorded after 7 days of treatment (Fig. 5.8). The results were presented in the Table 5.11

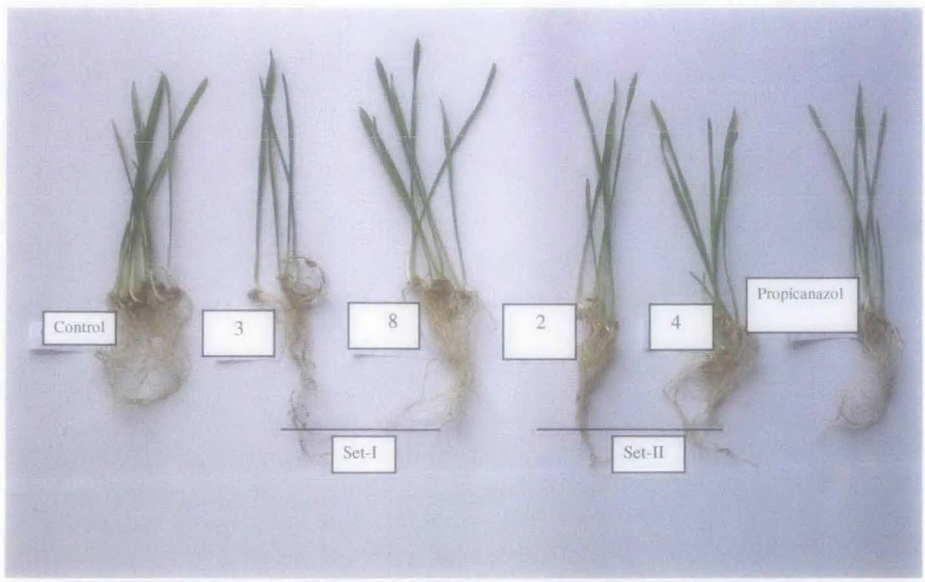


Fig. 5.8 Effect of organotin compounds on seedling growth.

Table 5.11: Effect of seed treatment with organotin compounds on seedling vigor of wheat (cv. Sonalika)

Set	Compound No.	Seed germination (%)[1]	Root length (cm)	Shoot length (cm)
I	**1.**	95.7	3.10	4.30
	2.	95.4	3.27	5.09
	3.	96.3	3.30	5.19
	4.	81.9	2.20	4.90
	5.	86.4	3.25	5.16
	6.	86.4	2.46	4.65
	7.	86.2	3.10	4.97
	8.	95.2	3.35	5.12
II	**1.**	92.4	3.16	5.02
	2.	94.5	3.21	5.15
	3.	93.2	3.23	5.21
	4.	87.5	3.02	4.95
	5.	83.4	2.94	4.72
	6.	88.6	2.74	4.62
	7.	89.5	2.85	4.75
	8.	89.5	3.01	4.99
	9.	83.4	2.92	5.01
	Control (Water)	95.5	3.32	5.27

[1]Seed germination percentage was recorded after 72 h. [2]Root and shoot length were recorded after 7 days.

It appears that the compounds shown effective against *B. sorokiniana* were also fungitoxic to *Alternaria titricina* and *Fusarium solani*. Among chemicals **2**, **3**, **5** and **8** in set I and **3**, **4**, **5** in set II almost completely inhibited spore germination of *Alternaria titricina* than other chemicals. These chemicals also inhibited spore germination of *Fusarium solani*. Though the extent of inhibition is slightly less, From the above result, it may be concluded that the newly synthesized organotin compounds have promise for the control of *Alternaria titricina* and *Fusarium solani* also.

It will be seen from the Table 5.11 that most of the compound either had no adverse or little effect on seed germination and its subsequent growth. Compounds like **4**, **5**, **6** and **7** in set I and **4**, **5**, **6**, **7**, **8** and **9** in set II caused mild inhibition of germination, recording 81-89% as compared to untreated check, recording 95% seed germination. Regarding root and shoot length, the result showed the same trend. No inhibition of seed germination was recorded with other chemicals like **1**, **2**, **3**, **8** in set I and **1**, **2**, **3** in set II even some chemical stimulated seed germination and its growth.

5.5.5 Effect of organotin compounds on *Alternaria titricina* and *Fusarium solani*

The compounds were further tested for their for their fungicidal effect on spore germination of *Alternaria titricina* and *Fusarium solani*, the two major pathogen of wheat in this agroclimatic region. For this purpose, seventeen chemicals found effective against *B. sorokiniana* were tested at 50 ppm concentration for their effect *in vitro* on spore germination tests and results were shown in Table 5.12

Table 5.12 Effect of organotin compounds on spore germination of *Alternaria titricina* and *Fusarium solani*

Set	Compound (50ppm)	Spore germination (%)	
		Alternaria titricina	*Fusarium solani*
I	**1.**	4.2	2.8
	2.	0	1.7
	3.	0	3.1
	4.	2.5	4.7
	5.	1.6	1.3
	6.	9.2	11.2
	7.	7.5	9.2
	8.	1.2	8
II	**1.**	3.1	5.1
	2.	2.7	7.5
	3.	0	6.2
	4.	1.2	8.5
	5.	0	7.5
	6.	2.1	4.6
	7.	7.5	8.9
	8.	6.4	10.6
	9.	8.2	12.5
	Control (Water)	85.2	89.6
CD (P= 0.05)		3.25	2.98

Results from various studies involving wheat-*B. sorokiniana* combination, clearly established the fact that newly synthesized organotin compounds have the potential for inducing significant level of resistance in wheat plants against foliar blight pathogen. The results of *in vitro* fungitoxicity assay make it clear that the compounds directly suppressed the pathogen. These compounds acted through host mediated response. Supports for this view came from the studies in the biochemical changes in the host following treatment with the compounds. As a result of the treatment induced change in the host metabolites involving significant change in the phenolics, proteins and substantial increase in the PO and PPO activities.

The biochemical basis of resistance of plants to fungal pathogen have been associated with infection induced antimicrobial compounds [175]. At present a large amount of information has accumulated on the possible role of phenolics in disease resistance. Matern and Kneusel [141] proposed rapid synthesis of phenolics following infection to be an important first line defense in the plants. The present investigation showed that organotin compounds enhance the biosynthesis of phenolics.

Changes in the host physiology following infection is often associated with an activation of oxidase activity and a post-infectional increase in the level of such enzyme is a common phenomena in the diseased tissue more so is an incompatible interaction. Activation of polyphenol oxidase activity would seem to be more important as it can oxidize phenolics to quinines which may be more fungitoxic [176]. In the present investigation increased polyphenol oxidase activity was associated with the resistance induced by the test chemicals. Present reports agree with the earlier reports on the association of stimulated PPO activity against *B. sorokiniana* [22].

Response of both untreated and variously treated plants in respect of PO activity followed almost the same trend as reported for PPO. Increased PO activity in response to infection is a common phenomena in many host parasitic interactions and the greater activity is generally linked with resistance response. The role of PO plant defense has been attributed to its PO activity to catalyse various types of oxidative reactions important in metabolism of pathogens or of the host plant such as phenolics, toxins, hormones etc. [177]. Increase activity of PPO, the terminal enzyme in phenyle propanoid pathway, appears to be associated with an increased synthesis and deposition of lignin at the site of infection [178].

In the present investigation, it has been clearly established that resistant plants have less disease against *Bipolaris sorokiniana* infection as such resistance are mostly correlated with an increased biosynthesis of phenolics and stimulated oxydase activity at and around the site of host-pathogen interaction. It is well known that phenylalanine ammonia lyase is the first enzyme of the phenyl propanoid pathway and considered as the key enzyme in the regulation of the flux of the phenyl propanoid compounds such as lignin and their derivatives and also appeared to be associated with hypersensitive reaction. In the present study, the resistant plants always had a higher PAL and peroxidase activities in comparison to susceptible

genotypes. The increased peroxidase activity is presumed to be associated with enhanced lignifications and also for production of phytoalexins.

Plants are known to respond to infection with both quantitative and qualitative changes in their protein content. In the present investigation, it was noticed that protein content increased initially in the infected tissue in treated and untreated plants due to infection by *B. sorokiniana*, though in case of treated plants it is more than that of untreated Sonalika cultivar. Synthesis of new protein in resistant cultivar following fungal infection has been reported in rice, wheat, tobacco and some other plants [147,179].

In summing up, the series of observation on foliar blight of wheat, it may be concluded that the newly synthesized organotin compounds act both directly and indirectly by conditioning the susceptible plant towards induced resistance through series of changes in the host metabolism bringing about activation and strengthening of the host innate defense potential so that the pathogen or its pathogenic activity or both are suppressed. Initial studies also indicate that these chemicals are fungitoxic to other pathogens like *Alternaria tritricina* incident of air borne leaf blight disease and *Fusarium solani* incident of soil borne foot rot disease in wheat, which is very significant.

5.6 References

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