BIOEFFICACY AND PERSISTENCE OF HEXACONAZOLE 5 SC AGAINST OKRA POWDERY MILDEW (*Erysiphe cichoracearum* DC) AND CHILLI POWDERY MILDEW (*Leveillula taurica* (Lev.) Arn.)

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COIMBATORE - 641 003

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Thesis submitted in part fulfillment of the requirements for the degree of

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2002

CERTIFICATE

This is to certify that the thesis entitled **"BIOEFFICACY AND PERSISTENCE OF HEXACONAZOLE 5 SC AGAINST OKRA POWDERY MILDEW (*Erysiphe cichoracearum* DC) AND CHILLI POWDERY MILDEW (*Leveillula taurica* (Lev.) Arn.) "** submitted in part fulfillment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a **bonafide** record of research work carried out by **MISS. P. MAREESWARI** under my supervision and guidance and that no part of this thesis has been submitted for the award of any degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

BIOEFFICACY AND PERSISTENCE OF HEXACONAZOLE 5 SC AGAINST OKRA POWDERY MILDEW (*Erysiphe cichoracearum* DC) AND CHILLI POWDERY MILDEW (*Leveillula taurica* (Lev.) Arn.)

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DEGREE : **DOCTOR OF PHILOSOPHY (Agriculture) IN PLANT PATHOLOGY**

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Studies were carried out to determine the bioefficacy of hexaconazole 5 SC against okra powdery mildew (*E. cichoracearum* DC.) and chilli powdery mildew (*L. taurica* (Lev.)Arn.) diseases. Persistence of the hexaconazole 5 SC, its dissipation rate, compatibility with insecticides and biocontrol agents, phytotoxicity, effect on spore germination, phylloplane and rhizosphere microorganisms, effect on host resistance, and harvest time residues in fruits and soil were also carried out.

Hexaconazole 5 SC, a triazole compound at four different concentrations *viz.*, 500 ml, 750 ml, 1000 ml and 1500 ml ha^{-1} was significantly effective against the powdery mildew disease in okra and chilli in three field trials and green-house trials. Increase in yield was observed in all the hexaconazole treatments and the efficacy of hexaconazole 5 SC enhanced with increase in its concentration. The compatibility studies indicated that the hexaconazole (500 ml ha^{-1}) was highly compatible with monocrotophos (1000 ml ha^{-1}) and showed its synergistic action on the pests and powdery mildew disease in okra and chilli. The persistence of hexaconazole 5 SC at all the concentrations was observed up to ten days after last spraying in okra fruits whereas the persistence of the hexaconazole at 1500 ml ha^{-1} in chilli fruits was observed up to seven days after last spraying. No phytotoxic effects were observed in all the concentrations of hexaconazole 5 SC. Hexaconazole at high concentration of 2000 ppm showed the least per cent spore germination of both *E. cichoracearum* and *L. taurica*.

Increase in chlorophyll content, total phenol and soluble protein content was observed in the leaves of okra and chilli due to hexaconazole treatment. The increased activity of defense related enzymes *viz.,* peroxidase, polyphenol oxidase and phenylalanine ammonia lyase was observed in the leaves of okra and chilli immediately after hexaconazole spray.

Spraying of hexaconazole 5SC resulted in increase in the phylloplane fungal and bacterial population with increase in hexaconazole concentration in okra and chilli but the fungal population in the phylloplane decreased with increase in day intervals. The rhizosphere fungal population in okra and chilli increased with increase in day intervals and reduced with increase in concentration. The rhizosphere bacterial population in okra increased in all the concentrations however there was no remarkable change in rhizosphere bacterial population in chilli.

The compatibility study of hexaconazole with biocontrol agents suggest that it was highly inhibitory to *Trichoderma viride* and *T. harzianum* even at the concentration of 1.0 ppm. However the growth of *Pseudomonas fluorescens* (Pf 1) and *Bacillus subtilis* was unaffected. Hexaconazole 5 SC at 1.0 ppm and 0.5 ppm was highly inhibitory to the pathogens *viz*., *Alternaria capsici* and *Colletotrichum capsici*. No residues of hexaconazole 5 SC was detected in the fruits of okra, chilli and in soil.

DEPARTMENT OF PLANT PATHOLOGY

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Thesis title : **BIOEFFICACY AND PERSISTENCE OF HEXACONAZOLE 5 SC AGAINST POWDERY MILDEW IN OKRA (*Erysiphe cichoracearum* DC.) AND CHILLI (*Leveillula taurica* (Lev.) Arn.)**

Key words : Hexaconazole, bioefficacy, persistence, powdery mildew, okra, chilli

Hexaconazole 5 SC at 500 ml, 750 ml, 1000 ml and 1500 ml ha^{-1} was highly effective against the powdery mildew disease in okra and chilli both in green-house and field experiments. Besides, the yield was also increased due to the test chemical spray. The test chemical was highly compatible with monocrotophos and mancozeb and its synergistic action was effective against the pests and powdery mildew disease. The persistence of the test chemical was observed up to ten days after third spray in okra fruits and it was up to seven days in chilli fruits at high concentration of 1500 ml ha^{-1}. No phytotoxic effect was observed in all the doses of hexaconazole 5 SC. The test chemical at 2000 ppm showed the least per cent spore germination of *E. cichoracearum* and *L. taurica*. The chlorophyll content, total phenol and soluble protein content were increased in okra and chilli leaves when these crops were sprayed with the test chemical. The increased activity of peroxidase, polyphenol oxidase and phenylalanine ammonialyase was observed and the induction of peroxidase and polyphenol oxidase

enzyme was also observed immediately after the hexaconazole spray. Spraying of hexaconazole resulted in increase in the fungal and bacterial population in the phylloplane and rhizosphere regions. The compatibility studies of hexaconazole with biocontrol agents indicate that it was highly inhibitory to *Trichoderma viride* and *T. harzianum* even at the concentration of 1.0 ppm. However the growth of *Pseudomonas fluorescens* and *Bacillus subtilis* was unaffected. Hexaconazole at 0.5 and 1.0 ppm was highly inhibitory to *Alternaria capsici* and *Colletotrichum capsici*. There were no residues of hexaconazole detected in the fruits of okra, chilli and also in soil.

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CHAPTER I

INTRODUCTION

India has emerged as the second largest producer of vegetables next to China with a production of over 72.83 million tonnes from a cropped area of 5.6 million ha in 1997-'98, of which Tamil Nadu produces 3.8 million tonnes from 0.15 million ha area (Manmohan Attavar, 2000). Okra (*Abelmoschus esculentus* Linn.) is cultivated throughout India as vegetable and also used in paper industry. The total area under okra cultivation in India was reported around 0.39 million ha during 1999-2000 (Khadar Basha and Bhaskara Reddy, 2001) and its production was 2.5 million tonnes. Chilli (*Capsicum annuum* L.) is an important vegetable cum universal spice crop grown throughout the year mainly for green chillies. India produces 1.02 million tonnes of green chilli from an area of 0.9 million ha, of which Tamil Nadu produces 0.04 million tonnes from an area of 0.06 million ha during 1999-2000 (Peter and Nybe, 2002).

One of the greatest challenges facing the world is to produce adequate food for the growing population. Under this circumstances, one third of the global food production is estimated to be destroyed annually by over 20,000 species of insects, diseases, weeds, mites, nematodes, rodents and other field storage pests (McEwen, 1978). Besides insects and weeds, plant diseases caused by fungi leading to yield loss to most of the crops in the field as well as under storage. Among the fungal diseases, powdery mildew disease is one of the most destructive diseases and become a major constraint in the production of most of the crops particularly, okra and chilli. The yield loss caused by powdery mildew disease in bhendi varies from 17.0 to 86.6 per cent (Jhooty *et al*., 1977).

Erysiphe cichoracearum DC, the powdery mildew pathogen of okra is causing the most widespread disease generally favoured by dry atmospheric and soil conditions, (Yarwood, 1957). Premature defoliation may occur as the fungus covers the leaf surface.

Yield reduction occurs in proportion to time and severity of disease development with late fruits often failing to mature and being small and misshapen (Walker, 1952). Powdery mildew fungi overwinter as active mycelial and conidial stages in the volunteer and cultivated okra plants. Heterothallism is reported for *E. cichoracearum* (Yarwood, 1935).

Leveillula taurica (Lev.) Arn., the major powdery mildew pathogen of Solanaceae family is prevalent in all dry areas. Disease affects mainly leaves and more rarely the stalks, flower parts or fruits. Chilli pepper (*Capsicum annuum*) in India is seriously affected (Mathur *et al*., 1972) leading to severe defoliation, reduction in size and number of fruits. *L. taurica* is easily distinguishable from other powdery mildews by the characteristics of endophytic mycelium and oidiopsis type conidiophores (Yarwood, 1978).

Control of plant diseases is indispensable to increase the fruit and vegetable production. The use of resistant varieties has been the principal method of controlling powdery mildew disease, but no source of complete resistance to powdery mildew is available in the cultivated bhendi (Jhooty *et al*., 1977). Of the various methods adopted, fungicides are undoubtedly the major and the only practical means of effective and reliable control of plant diseases. Elemental sulphur or compounds containing sulphur have long been known to have activity against mildews. However many of these fungicides are predominantly protectant. In recent years, the situation has changed due to development of very active fungicides like benzimidazoles, ethirimol (Bebbington *et al*., 1969) and triadimefon (Frohberger, 1973) and ergosterol biosynthesis inhibitors (EBIs) which constitute an important group of systemic fungicides for the control of powdery mildew. Most EBIs interfere with the biosynthesis of ergosterol by inhibiting C_{14} demethylation (Sisler *et al*., 1983). Time of application is important because differences in date of application may very much alter the efficiency of disease control and enhanced the yield (Bainbridge and Jenkyn, 1976).

Among the triazoles, hexaconazole is a new highly active, broad spectrum fungicide synthesised at Jealott's Hill Research Station of Plant Protection Division of ICI Agrochemicals (Shephard *et al*., 1986) which is sold under the trade name of Anvil and Planete (Smith, 1991). The new fungicide hexaconazole needs to be tested for its compatibility with insecticides to reduce the cost, labour and time of application (Hewlett, 1961). Persistence of protective fungicides on the host surface plays an important role in determining their disease controlling potential and useful in developing spray schedules (Thind and Jhooty, 1982).

Since the use of biocontrol agents forms an important component of integrated disease management, the knowledge on compatibility of fungicides with biocontrol agents is necessary to formulate the integrated disease management strategies. Being the systemic fungicide, hexaconazole may alter the beneficial microbes of both phylloplane and rhizosphere ecosystem and this may result in the disturbance or unpredictable changes in host and pathogen activity. Hence the effect of hexaconazole needs to be tested both in the phylloplane and rhizosphere region.

Fungicides act as chemical and abiotic components involved in induced systemic resistance in plants against pathogens and reduce the disease severity (Davidse and Ward, 1984). These fungicides interact with the plant constituents after spraying and cause quantitative and qualitative changes (Kotastane and Vyas, 1992). Fungicides application results in biochemical changes in plants is important to investigate the effectiveness and mode of action of the chemical against pathogens.

Fungicides are successful in controlling plant diseases but their excessive, irrational and indiscriminate use can pose problems pertaining to the safety of consumers. There may be serious residue problems especially when these are applied at the maturing

stage. As many of the fruits and vegetables are consumed as raw products, fungicide residues on them may lead to health problems. The residue levels in the soil or edible parts vary with the dose of the fungicides used and with total number of sprays done (Tripathi *et al*., 1976; Mithyantha *et al*., 1977).

Residues in harvested produce/fruits pose serious health hazards to human beings (Mott and Snyder, 1987) and mammals (Khera, 1987). Hence there is a need to detect the presence of residues in the harvested produce well in advance before it is available for consumption or export to developed countries. With this background, the following objectives were designed throughout the investigation.

1. To study the bioefficacy of hexaconazole 5SC against okra and chilli powdery mildew under green-house and field condition.
2. To study the persistence, dissipation and phytotoxicity of hexaconazole 5SC in okra and chilli fruits.
3. To study the induced systemic resistance in okra and chilli due to the spray of hexaconazole.
4. To assess the effect of hexaconazole on phylloplane and rhizosphere microbial population.
5. To test the compatibility of hexaconazole with insecticides and biocontrol agents and
6. To determine the hexaconazole residues in the harvested fruits of okra and chilli.

CHAPTER II

REVIEW OF LITERATURE

Chilli and okra crops are affected by the powdery mildew disease, the most destructive disease of both the crops resulting in economic yield losses. The role of various fungicides in control of the disease has been reported across the globe. The literatures pertaining to the disease control by fungicides, persistence of fungicides in plants, their compatibility with insecticides and biocontrol agents, non-target effect of fungicides, spore germination, their role in induction of systemic resistance, effect on biochemical constituents of plants and their residues in plant parts are reviewed in this chapter.

2.1. Powdery mildew disease of okra

Powdery mildew disease of okra caused by *Erysiphe cichoracearum* DC (Sub division: Ascomycotina; Order : Erysiphales; Family : Erysiphaceae) is prevalent throughout the okra growing areas of Tamil Nadu. The fungus is known to produce the oidial or conidial stage as well as the perfect stage. They produce conidiophores almost at right angle to the host surface. The conidiophores are hyaline, thin-walled and bear chains of conidia which are hyaline, thin-walled, oblong and measuring 24-30 x 15-20 µ. The cleistothecial stage is formed later on as the disease advances. The cleistothecia are dark coloured, spherical with thick-walled appendages. They contain numerous asci which are sub-cylindric. Each ascus contains two or three ascospores, which are hyaline, elliptic and thin walled. No source of complete resistance to powdery mildew is available in the cultivated okra (Jhooty *et al*., 1977).

2.2. Powdery mildew disease of chilli

Powdery mildew disease of chilli caused by *Leveillula taurica* (Lev.) Arn. (Sub division : Ascomycotina; Order : Erysiphales; Family : Erysiphaceae) is common in chilli growing areas of India. It attacks the leaf covering it with powdery whitish growth on both the surface causing premature defoliation. The fungus is air-borne, spreading from one field of chilli to another and from one host species to another in the vicinity when favourable climatic conditions prevail (Rangaswami and Mahadevan, 1999). *Leveillula taurica*, one of the causal agents of powdery mildew in solanaceous and other plants was identified for the first time in Lithuania on single damaged aubergine (*Solanum melongena*) leaves and its symptoms are briefly described (Grigaliunaite, 1999).

2.3. Chemical control of okra and chilli powdery mildew

2.3.1. Okra powdery mildew disease (*Erysiphe cichoracearum*)

Regupathy and Thamburaj (1990) reported that among the five fungicides tested against okra powdery mildew caused by *E. cichoracearum*, tridemorph (0.05%) proved to be the most effective in controlling the disease and recorded the highest green pod yield of 7634 Kg ha^{-1}. Gawande and Peshney (1987) reported that spraying of carbendazim (0.2%) once or tridemorph (0.08%) followed by dusting with sulphur twice at 10 days interval was found effective in controlling the powdery mildew. Dhurj *et al*. (1994) noticed considerable reduction in bud infection of powdery mildew with hexaconazole in rose. Chandrashekar and Sharma (1996) reported that hexaconazole (0.1%) was found to be highly effective over various other fungicides *viz.,* thiophanate methyl, triadimefon and wettable sulphur when used against powdery mildew of peas.

2.3.2. Chilli powdery mildew disease (*Leveillula taurica*)

Patel *et al*. (2000) reported that a field trial was conducted in Gujarat during 1995 to control powdery mildew (*Leveilula taurica*) with different systemic and non systemic fungicides (Penconazole, carbendazim, thiophanate methyl, tridemorph, wettable sulphur, sulfur dust, dinocap and triadimefon). All the fungicides were significantly effective. Among these, 0.05 per cent carbendazim was the most effective (2.27% disease intensity) also recorded the highest yield (4120 kg ha^{-1}) as against control (9.30% disease intensity).

Thiophanate methyl, triadimefon and tridemorph were more effective than remaining fungicides. Wettable sulphur, sulfur dust and dinocap recorded 50 per cent disease control. Systemic fungicides were more effective than non systemic fungicides.

2.3.3. Triazole group of fungicides

Triazoles are registered agrochemicals, highly effective which are broad-spectrum products. Used as seed treatment and foliar fungicides. Triazole fungicides are coming under the azole class of sterol biosynthesis inhibitors grouped under systemic fungicides. Triazoles exert their fungitoxic effects by inhibiting the 14 α-methyl sterol 14 α-demethylase enzyme system involved in sterol biosynthesis (Kato 1986; VandenBosch, 1988) which is a cytochrome P-450 dependant monooxygenase.

The triazole compounds possess a ring composed of two carbon atoms and three nitrogen atoms. A systemic fungicide RH-1,2,4 (4-n-butyl-1,2,4-triazole, BT) has been introduced as an experiment fungicide, possessing high selective activity against brown rust of wheat (Waller *et al.,* 1990). The substitution on the nitrogen atom in position 1 in the triazoles producing amazing variety of compounds such as triadimefon, triadimenol and biloxazol.

Triadimefon is volatile and reduced within plants to form triadimenol. While both forms are fungitoxic, the fungitoxicity of triadimefon depends on the rate at which fungi formed triadimenol which is an active principle of toxicity. The third compound biloxazol with additional phenyl group is more lipophilic and less systemic. Tricyclazole, dichlobutrazole, epoxiconazole, difenoconazole and tebuconazole were included recently in triazole group of fungicides. The triazole group of fungicides are listed in Table 1.

2.3.3.1. Mode of action of triazole fungicides

The basic mode of action of triazole fungicides is the inhibition of the biosynthesis of ergosterol, the principal sterol in the membranes of most fungi (Sisler *et al*., 1983). The primary action of Sterol Biosynthesis Inhibitors is blocking of the conversion of lanosterol to ergosterol which occurs in two different ways: the first group of inhibitors (including azole fungicides) affect the 14-α-demethylation of lanosterol and 24-methylene-dihydrosterol, whereas the second group (morpholines) block the conversion of fecosterol to episterol (Bloch and Mercer, 1987).

The C_{14} demethylation is the most sensitive site (Buchenauer, 1987) for the action of sterol biosynthesis inhibitors. Mercer (1984) found that in the first step, 14-α-methyl group (CH_3) is oxidised to a 14 α-hydroxymethyl group (CH_3OH); hydroxymethyl group is oxidised to a formyl group (CHO) and finally the formyl group is oxidised and lost as formate (HCCOH) along with the 15 α-hydrogen. These steps are catalysed by mono-oxygenase catalysing enzymes, the first step is catalysed by cytochrome P-450, while other steps are not.

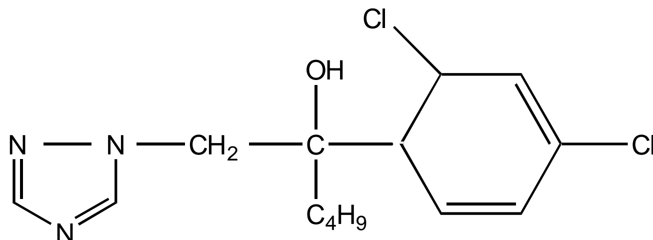
Buchenauer (1987) reported that the absence of oxygenated intermediates during the C_{14} demethylation reactions indicate that the first oxygenation step which is catalysed by a cytochrome P-450 enzyme is the primary site of action of sterol biosynthesis inhibitors.

2.3.3.2. Hexaconazole - a novel protectant curative triazole fungicide

Among the triazoles, hexaconazole [(RS)-2-(2,4-dichlorophenyl)-1-(1H-1,2, 4-triazol-1-yl) hexan-2-ol] is a new highly active, broad spectrum fungicide synthesised at the Jealott's Hill Research Station of Plant Protection Division of ICI Agrochemicals (Shephard *et al*., 1986) which is sold under the trade name of Anvil and Planete (Smith, 1991). The fungicide has broad spectrum antifungal activity and low mammalian toxicity. Its outstanding protectant activity combined with curative, translaminar, antisporulant and systemic properties enable it to be used effectively against diseases of many crops at very low application rate.

2.3.3.3. Properties of hexaconazole

Structural formula :



Hexaconazole

Molecular formula	:	$C_{14}H_{17}Cl_2N_3O$
Molecular weight	:	314
Appearance	:	White crystalline solid
Melting point	:	111°C
Density	:	1.29 g/cm^3 at 25°C
Vapour Pressure	:	2 x 10^{-8} KPa at 20°C
Solubility (g/l at 20°C)	*:*	*Water 0.017*
		Methanol 246
		Acetone 164

Toluene 59

Hexane 0.8

Stability : Stable for atleast 9 months at ambient temperature

2.3.3.4. Toxicology of hexaconazole

Hexaconazole has low mammalian toxicity and is readily excreted by mammals with no significant retention in organs or tissues. It is classified as a mild eye irritant, but non-irritant to the skin. It is not mutagenic. Also it is less toxic to birds, fish, bees and other wild life species. Residues of hexaconazole in crops treated at recommended rates have been very low (less than 0.01 to 0.03 mg/Kg) and degradation in laboratory soil is rapid and mobility through soil was low (Shephard *et al*., 1986).

2.3.3.5. Bioefficacy of hexaconazole against plant pathogens

Hexaconazole has a broad spectrum activity against ascomycetes and basidiomycetes fungi (Worthington, 1991). The efficacy of hexaconazole fungicide against various pathogens in different crop species has been listed in Table 2.

Varalakshmi *et al*. (1999) reported that hexaconazole at a higher concentration of 0.2 per cent was effective against powdery mildew disease in grapes and it recorded the disease reduction of 76 per cent over untreated control. The yield was maximum (12.0 Kg/ha) at 0.2 per cent hexaconazole treatment followed by the same fungicide at 0.1 and 0.05 per cent.

Combination of the systemic fungicide hexaconazole with therapeutant captan, a new formulation of hexaconazole (Contaf 5 SC) was evaluated against powdery mildew disease of rose, indicated that the combination of hexaconazole and captan performed

very well and provided superior control of the disease over Bayleton 25 WP, Tilt 25 EC and Contaf 5 SC (Shitole *et al*., 2000b). Hexaconazole offered excellent control of soybean rust (*Phakopsora pachyrhizi* Syd.) disease (Senthil *et al*., 2000).

2.4. Persistence of triazole fungicides in plants

Persistence of protective fungicides on the surface of the plant /plant parts plays an important role in determining their disease reduction potential and is useful in developing spray schedules (Thind and Jhooty, 1982). Work done on residues of fungicides in India is meagre although a number of fungicides are being used at present. The residue levels in the soil or edible parts vary with the dose of the fungicides used and with total number of sprays done (Tripathi *et al*., 1976; Mithyantha *et al*., 1977).

Residues of hexaconazole (Anvil ® 5 SC) in the samples of grapes initially were more in samples collected on 0th day after spraying in both the concentration *viz.,* 25 g a.i ha^{-1} and 50 g a.i ha^{-1} recorded 0.60 and 0.61 ppm respectively. Later as the time interval increased, the residues in the samples decreased and it is below detectable level from 7th and 10th day after spraying with 25 g a.i ha^{-1} and 50 a.i ha^{-1} respectively (Varalakshmi *et al*., 1999).

Hexaconazole was more persistent in all soils under flooded conditions than under non-flooded conditions and at 27°C than at 35°C. The soil persistence of hexaconazole was not affected by the addition of wheat straw both under flooded and non-flooded condition (Neera Singh and Dureja, 2000).

The fungicide residues on grapes (cyproconazole, hexaconazole, kresoximmethyl, myclobutanil, penconazole, tetraconazole and triadimenol), the application rates of which were of a few tens of grams per hectare were very low after treatment and were not detectable at harvest (Cabras and Angioni, 2000).

2.4.1. Persistence of other group of fungicides in plants

Thind and Jhooty (1982) reported that though the initial deposition of dithane M-45 on all the tomato cultivars was almost the same, its persistence varied on different cultivars and it was persisted for longer period in glass house than in the field. Gajbhiye *et al*. (1995) reported that the residues of flusilazole on apple fruits became non-detectable (<0.001 ppm) within 15 days of application at 40 and 80 g / ha rate of application with a half-life of 2.1 to 2.8 days. A safe waiting period of 4 days was recommended for commercial use of flusilazole.

The persistence of all the three formulations of mancozeb (Dithane M-45, Dhanuka M-45and Luze M-45) was more in apple leaves (24 days) as compared to fruits (20 days), whereas the Dhanuka M-45 and Luze M-45 persisted only upto 20 and 16 days in leaves and fruits respectively (Gupta *et al*., 1994).

The residues of two ethylene bisdithio carbamates (DM-45 and DZ-78) at two concentrations *viz*., 0.25 and 0.5 per cent on Anab-e-shahi ranged from 26 to 44 ppm and 28 to 35 ppm for Dithane M-45 and DZ-78 respectively at 0.5 per cent concentration (Bhupal Reddy *et al*., 1993).

Fenarimol residues persisted in grapes to the maximum of 14 days after last spray that too at very low level (Balamuralikrishnan and Jeyarajan, 1998). Samples of apple and grapes obtained from local markets in and around Delhi contained both EBDCs and ETUr residues and the presence of ETU in all the samples analysed was however of great concern (Udaykumar and Archanakumari, 1998).

The dodine fungicide residues persisted in citrus leaf for a period of 15 days after the last spray (Ray and Addy, 1976) and guava fruits (Agnihotri *et al*., 1985) up to a period of 15 days and up to a period of 30 days in apple fruits (Agnihotri *et al*., 1985).

Sharma *et al*. (1997) reported that the initial deposits of carbendazim were lower in apple fruits than in leaves. However, the rate of degradation was similar on fruits and leaves up to the last day of analysis. On fruits, the residues could be detected up to 10 days whereas on leaves, these even persisted beyond this period.

Rane *et al*. (1997) reported that the residue levels in peel and pulp of mango fruits recorded one day after treatment were 1.60 to 1.89 and 0.46 to 0.98 ppm respectively. Residue level was reduced gradually in samples drawn from 3 to 10 days.

Metalaxyl persisted for 15 days in leaves and 9 days in roots of opium when applied on foliage (Anila Doshi and Thakore, 1995). The residues of carbendazim on cauliflower were 1.85 ppm and 0.71 ppm with 500 g ha^{-1} and 250 g ha^{-1} doses used respectively (Agnihotri *et al*., 1988). With three sprays of Dinocap @ 0.625 g product L^{-1} at 7 days interval for control of powdery mildew on greenhouse cucumber, it is reported that the mean residue to fall below the permitted level of 0.1 $\mu g\ g^{-1}$ 3 days after the last spray

(Ripley *et al*., 1985). The residues of dodine at 3.0 and 6.0 Kg a.i. ha^{-1} on apple fruits and leaves could be detected upto 20 d on fruits and 30 d on leaves (Sharma *et al*., 1996).

The residual effect of systemic and non-systemic fungicides on seed and other plant parts was studied for varying periods against *Alternaria alternata* (Fr.) Keissler causing leaf blight of tomato. Seed treatment with different fungicides showed persistence of carbendazim for 90 days. Non-systemic fungicides applied on foliage persisted for 10 days. Systemic fungicides like Aliette persisted in roots and leaves of tomato seedlings upto 23 days while thiophanate methyl and carbendazim remained for 19 days after soil application (Chandravanshi *et al*., 1994).

2.5. Compatibility of fungicides with insecticides / herbicides

Insecticides and fungicides are being applied separately for the control of pest and diseases. This becomes rather costly and also involves more of labour and time in applying If insecticides and fungicides are applied simultaneously in a single operation it could be cheaper in farmer's point of view. But as the fungicides and insecticides are chemically different in nature, their compatibility may pose a problem and hence thorough investigation is required before its widespread application.

Mixture of two pesticides may produce a greater insecticidal action than the sum of their individual components by exhibiting synergism (Gera, 1973), thus minimising the pesticidal load on the environment. Mixtures may also bring about significant cost efficiency (Hewlett, 1961).

The compatibility studies of Varalakshmi *et al*. (2000) showed that the haxaconazole + monocrotophos (500 ml ha^{-1} + 1000 ml ha^{-1}) combination was highly effective over other treatments in reducing the powdery mildew incidence, thrips and flea beetle damage in grapes. Both the fungicide and insecticide were found to have synergistic effect and exerted high efficiency towards pests and disease of grapes. Padmaja and Kameshwara Rao (2000) reported that combination of fenvalerate 0.01 per cent and mancozeb 0.1 per cent recorded the highest percentage mortality of *Spodoptera litura* larvae. Similarly monocrotophos 0.05 per cent + carbendazim 0.05 per cent, carbaryl 0.1 per cent + mancozeb 0.1 per cent were highly compatible and recorded high mortality than their individual insecticidal spray. Similar results were observed in the control of *Drosophila melanogaster* by fenvalerate + carbendazim (Reddy, 1984) and pea pod borer control by monocrotophos and carbendazim (Shukla and Lal, 1989).

The influence of herbicides on the antifungal activity of some fungicides with *Phytophthora* blight of pigeon pea was studied by Birendra Singh *et al.* (1999). Two pre-emergence herbicides enhanced the *in vitro* toxicity of the fungicides Apron 35 WS and Ridomil MZ-72 against the growth of *P. drechsleri f. sp. cajani* but reduced the toxicity of captafol. The combination of herbicides fluchloralin and alachlor with carbendazim, benomyl and carboxin altered their fungicidal action and showed synergistic effect against *Fusarium oxysporum, Sclerotium rolfsii, A. brassicicola and Colletotrichum capsici* (Reddy and Vir, 1991). Fluchloralin and alachlor reduced the toxicity of methoxyethyl mercury chloride (MEMC) and propanocarb against *Pythium butleri* and with carbendazim against *R. solani in vitro* (Kataria and Dodan, 1982).

2.6. Compatibility of fungicides with biocontrol agents

The indiscriminate use of potentially hazardous fungicides pose a serious threat to environment. The build up of resistance by the pathogen and the non-target effect of fungicides on beneficial organisms such as nitrogen fixers, residential antagonists and mycorrhizal fungi are the other disadvantages of the application of fungicides (Rodriguez-Kabana and Curl, 1980). Since the use of biocontrol agents forms an important component of integrated disease management, the knowledge on compatibility of fungicides with biocontrol agents is necessary to formulate the integrated disease management strategies. *Trichoderma harzianum* application with synthetic fungicides gives reliable control of *Botrytis cinerea* (Elad *et al*., 1993) reduces chemical inputs and eases the risk of fungicide resistance.

Based on the *in vitro* experiments, Baicu (1982) classified the fungicides and insecticides into three groups according to their toxicity level to the biological control agent *Trichoderma viride.*

Group I – Substances which were non-toxic both to spores and to mycelia : Permethrin (Ambush), deltamethrin (Decis), fenvalerate (Sumicidin), kelevan (Despirol), carbaryl 50 per cent, carbaryl + gamma – HCH (Lindane), dimethoate (Sinoratox), trichlorfon (Dipterex), diazinon (Basudin), ethion (DEF-25), copper oxychloride (Turdacuprul), wettable sulphur, quinomethionate (Morestan), dinocap (Karathane) and folpet (Ortho phaltan).

Group II - Substances which were moderately toxic to spores and mycelia or toxic either to spores or to mycelia : captan 50 per cent, barium polysulphide, polychlorpinene (Pinetox), malathion (Carbetox 37), mancozeb (Dithane M-45) and zineb (Perozine).

Group III - Substances which were toxic to spores and mycelia : dithiocarbamic complex (PET-183), copper hydroxide, benomyl (Benlate), fenarimol (Rubigan), nuarimol (Trimidal), trimorfamide (Fedemorf) and thiophanate methyl (Metoben).

The pesticides included in group I and some of those in group II could be used successfully in integrated control systems. The fungicides in group III were highly active against the antagonistic fungus, *Trichoderma viride* and were not included in integrated disease management.

Howell and Stipanovic (1994) reported that the sterol production inhibitors like propiconazole or flusilazole at the rate of 1 $\mu g\ ml^{-1}$ and myclobutanil or triadimenol at 1 to 4 $\mu g\ ml^{-1}$ suppressed viridiol production without affecting the growth of *Gliocladium virens* on potato dextrose agar medium. But at increased concentration, the chemicals highly inhibited the mycelial growth of *G. virens*.

Selected isolates of *Trichoderma spp*. (two isolates of *T. harzianum* and one each of *T. viride, T. reesei* and *T. koningii*) when tested with Captaf 500 ppm, Dithane M-45 at 500 ppm and thiram at 200 ppm, the fungicides were highly inhibitory to *T. reesei* and they were compatible to *T. koningii*. Thiram at 200 ppm inhibited *T. viride* while the other two fungicides were compatible with *T. viride* (Singh *et al.,* 1995).

Sharma and Misra (1995) reported the tolerance of *T. harzianum* to various agrochemicals. The fungicides metalaxyl, chlorothalonil and captafol showed little inhibition while thiram was highly inhibitory even at lower concentration. The insecticides

aldicarb, phorate and carbofuran were less toxic. The nematicides Ethoprop and Subufos were less toxic while vapam and phenamiphos were highly toxic.

The benzimidazole fungicides, *viz.,* carbendazim and benomyl were toxic to the rice sheath blight pathogen *R. solani* as well as the antagonists *viz., Gliocladium virens, T. longibrachiatum* and *T. harzianum,* while the organophosphorus fungicides, edifenphos and iprobenphos were more toxic to the pathogen alone. However, these fungicides were also inhibitory to antagonists at high concentrations (Viji *et al.,* 1997). Similarly the deleterious effect of benzimidazole group of compounds were also observed by Backman and Rodriguez-Kabana (1977) and Papavizas *et al.* (1982).

Dubey (2000) also recommended the combined use of fungicides and biocontrol agents for the management of web blight caused by *R. solani* in groundnut. The seeds treated with *Gliocladium virens* in integration with Thiram gave maximum seed germination, minimum disease incidence with highest yield followed by *G. virens* alone treated seeds. The viability of entomopathogenic fungus, *Metarrhizium anisopliae* in mixture with agrochemicals was studied as a part of IPM programme against coffee pest, *Hypothenemus hampei*. Among the various fungicides tested, cyproconazole, hexaconazole and triadimefon were fungitoxic to *M. anisopliae* (Gonzalez *et al.,* 1996).

2.7. Effect of fungicides on non-target microorganisms

The systemic fungicides which are introduced into the environment and soil for the control of plant diseases can be expected to result in a diverse array of effects on target and non-target organisms. Systemic fungicides have a wide range of biological activity. The different organs of a plant support a range of microorganisms. If it is on the

leaf, they are named as phylloplane microbes and if it is in the root, they are said to be rhizosphere microorganisms.

Systemic fungicides may alter the microbes of both phylloplane and rhizosphere ecosystem because their action is rarely limited to the specific organism they are intended to control. If the microflora are altered, the interactions among their component groups including phytopathogenic fungi and bacteria may change. If antagonists to a pathogenic microorganism are inhibited by a fungicide, the activity of that microorganism could increase. The study on the effect of systemic fungicides on the alterations of microorganisms is very much important for disease management (Viji *et al*., 1997).

2.7.1. Effect of fungicides on phylloplane and rhizosphere microflora

The phylloplane microflora is determined by the interplay of many factors, including fungicides. Several fungicides reduce saprophytic population of microorganisms in the phylloplane of crops. Dik (1990) reported that a reduction in the population of yeast due to fungicide application on grapes may also affect the fermentation of grape juice. The increased infection after benomyl application by pathogens tolerant to this fungicide on cereal phylloplane (Fehrmann, 1981) and rye phylloplane (Fokkema *et al.,* 1975) was due to reduction of the saprophytic microflora of phylloplane to a level which was no longer antagonist.

In wheat, phylloplane fungi were isolated and tested against benomyl 40 ppm. Nine species of *Cochliobolus,* 14 species of *Drechslera,* 4 species of *Helminthosporium* and five species of *Pyrenophora* showed tolerance towards benomyl (Greenway, 1973). The late application (before or after anthesis) of carbendazim (0.15%) and tridemorph

(0.05%) reduced the saprophytic microflora on phylloplane and increased the yield up to 21 per cent (Fokkema and de Nooji, 1981). One or two applications of benomyl to wheat disturbed the normal fungal flora. An average fall of 30 per cent in the fungal population was observed by Skajennikoff and Rapilly (1981). Metalaxyl, the systemic fungicide applied for the control of blue mold of tobacco (*Peronospora tabacina*), reduced the phylloplane microflora of tobacco by 20.7 to 33.6 per cent (Duccoman and Corbez, 1982).

The systemic fungicides after absorption through roots and leaves move acropetally to the apical region and accumulate in the periphery of the leaves and are excreted (Heuvel, 1979). These excretions are toxic to the phylloplane microorganisms. Gross and Kenneth (1973) observed that benomyl and thiabendazole inhibited *Sporobolomyces roseus, Leucosporium scotii* and *Aureobasidium pullulans in vivo* and *in vitro*. Bollen and Scholten (1971) reported that the strains of *Botrytis cinerea* has a large degree of tolerance to benomyl, hence caused severe head rot incidence in cyclamen after the treatment. After some time, the disease incidence was reduced again to a normal level.

The fungicides MBC and benomyl drastically reduced the nitrogen fixing bacteria *Corynebacterium spp*., *Azotobacter chroococcum* and *Bacillus spp*. when applied on wheat, rice and jute respectively (Pati and Chandra, 1981). Fokkema and deNooji (1981) reported that broad spectrum fungicides such as captan and dithiocarbamates were not inhibitory to bacteria.

2.8. Effect of fungicides on spore germination

Studies on the *in vitro* efficacy of fungicide against the spore germination of the pathogen revealed that even at a concentration of 100 ppm, it was able to reduce the

spore germination up to 38.9 per cent. With the increasing concentration of the chemical, the number of spores germinated reduce revealing the increased sensitivity of *Uncinula necator* to the concentration of hexaconazole (Varalakshmi *et al.,* 1999).

Trifloxystrobin (0.1 $\mu g\ ml^{-1}$) was very effective against the conidial germination of *Uncinula necator* under *in vitro* condition and it inhibited the mycelial growth and sporulation (Reuveni, 2001). Azoxystrobin, a systemic broad spectrum fungicide of the new strobilurin group, inhibits spore germination and mycelial growth in a wide range of plant pathogenic fungi (Beaumont, 2001).

2.9. Role of chemicals / fungicides / herbicides in Induced Systemic Resistance

The classical inducers of Induced Systemic Resistance (ISR) can be grouped as either abiotic or biotic. Abiotic inducers include salicylic acid, dichloro isonicotinic acid, jasmonic acid, aminobutyric acid, plant extracts, phosphates, herbicides, fungicides, plant growth regulators etc. and biotic inducers are pathogens, avirulent pathogens, non-pathogens, elicitors from pathogens and plant growth promoting bacteria.

The activity of peroxidase, polyphenol oxidase, phenylalanine ammonialyase, tyrosine ammonialyase and catechol-o-methyl transferase were higher in the probenazole treated and *P. oryzae* inoculated leaves when compared to the untreated and/or inoculated leaves. This indicated that the disease controlling mechanism of probenazole was due to the host-mediated defense reaction (Davidse and Ward, 1984).

Similarly application of Fosetyl-Al to the tomato leaves infected by *Phytophthora capsici* resulted in accumulation of the phenolic compounds, phytoalexins and phenylalanine ammonialyase enzyme (Bompeix *et al.,* 1981) at high concentration in tomato.

The herbicide dinitro aniline induced systemic resistance against fusarial wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* in tomato (Starratt and Lazarovits, 1996). Oostendrop *et al.* (1996) reported that triazole fungicides like epoxiconazole and propiconazole induced systemic resistance against *Colletotrichum lagenarium* in cucumber. The probenazole treatment for *Xanthomonas oryzae* pv. *oryzae* management resulted in the accumulation of PR-protein, heat shock protein and thylakoid protein in rice leaves (Lalithakumari and Dhakshinamurthy, 1995). Different isoforms of chitinases were induced from pepper stems treated with mercuric chloride (Kim and Hwang, 1996).

2.10. Fungicides causing biochemical changes

There are several reports on the biochemical changes in plants after fungicide treatment as foliar spray and soil drenching. The triazole chemical treated plants typically appear dark green than untreated control. This change was correlated with increased chlorophyll content (Fletcher *et al.,* 1986; Sankhla *et al.,* 1985). Similar results were also observed in *Poa pratensis* leaves treated with triazole chemicals (Kane and Smiley, 1983). Lambers (1982) observed increased carbohydrates in roots of paclobutrazol treated plants. Similar trend was also found in bottlegourd leaves sprayed with hexaconazole (Rajveersingh and Thakore, 1998). Increased chlorophyll content was observed in cucumber seedlings treated with triazole fungicides such as uniconazole, bitertanol, diniconazole and hexaconazole (Lee *et al.,* 1999).

Carbendazim application resulted in increased accumulation of total phenol in groundnut (Singh and Kang, 1978), tomato (Karadge and Karne, 1986) and chilli plants (Sharma *et al.,*1990).

Carbendazim and Vitavax as seed treatment and soil drenching significantly reduced the dry root rot of chick pea with corresponding increase in phenolic and carbohydrate content (Rajindersingh and Sindhan, 1998). Kotastane and Vyas (1992) also reported the similar observation in mustard after carbendazim, mancozeb and zineb application as foliar spray, seed treatment and soil drenching.

2.11. Fungicide residues in harvested produce

The effectiveness of fungicides against the disease may lead to indiscriminate use which in turn could leave higher levels of their residue in harvested produces. It has been documented that the residue of dithiocarbamates pose serious health hazards to human beings (Mott and Snyder, 1987). The mancozeb (manganese ethylene bisdithiocarbamate (polymeric) complex with zinc salt) has come under close scrutiny of health protection agencies because of its major metabolite ethylenethiourea (ETU) which has carcinogenic, teratogenic and goitrogenic effects on mammals (Khera, 1987). Rats developed thyroid cancer due to the intake of ethylene thiourea (Ulland *et al.,* 1972). Because of these problems, FAO is recommending a Maximum Residue Limit (MRL) for hazardous chemicals like insecticides, fungicides, herbicides, drugs for human being etc.

The fungicide residues in the harvested produce were at below detectable level for majority of the fungicides. The hexaconazole residues were at below detectable level in grapes (Heaney *et al.,*1986; Varalakshmi *et al*., 1999) and soybean (Senthil *et al.,* 2000). Similarly the tebuconazole residues were at below detectable level (BDL) in wheat grains

(Srivastava *et al.,* 1997). Sarkar *et al.* (1998) observed that the fungicide, Antracol was at BDL in potato and cropped soil. Similar results were obtained by Kalpana Diwan *et al.* (1999) in groundnut kernel, fodder and shell.

The FAO and WHO jointly conducted meeting on pesticide residues during 1990 and proposed the maximum residue limits (MRL) of hexaconazole as 0.5 mg Kg^{-1} for wheat straw and dry fodder, 0.1 mg Kg^{-1} for apple, grapes and wheat and 0.05 mg Kg^{-1} for banana, coffee beans and apple juice.

Residues of hexaconazole in the harvest samples of soybean for three seasons was studied by Senthil *et al*. (2000) and reported that hexaconazole at 0.2 per cent treated plots had an end residue of 0.02 ppm and in seed kernal, oil and deoiled cake samples, the residues reached below detectable level (BDL) at harvest.

Table 1. Triazole group of fungicides

S. No.	Common name	Trade name	Diseases/Pathogens	Reference
1.	Biloxazol (bitertanol)	Baycor Sibutol	Fungi imperfecti	Kraus, 1979
2.	Cyproconazole	-	Powdery mildews	Gisi *et al.*1986
3.	Dichlobutrazole	Vigil	Powdery mildews	Bent and Skidmore, 1979
4.	Diniconazole	-	Smut, Bunt	Takuno *et al.* 1983
5.	Etaconazole	Vangard Sonax	*Septoria spp.*	Staub *et al.*1979
6.	Fluotrimazole	Persalon	Powdery mildew in fruits and ornamentals	Grewe and Buchel, 1973
7.	Flutriafole	Impact Ferrax	Powdery mildew in fruits and ornamentals	Skidmore *et al.* 1983
8.	Flusilazole	Nustar Punch	Scab, Powdery mildew	Moberg *et al.* 1985
9.	Furconazole	-	Powdery mildew	Zech *et al.* 1988
10	Hexaconazole	Anvil	**Alternaria**	Shephard *et al.*1986
11.	Mycobutanil	Sisthane	Scab	Orpin *et al.* 1986
12.	*Penconazole*	Topas	**Geotrichum**	Eberle *et al.* 1983
13.	Propiconazole	Tilt Desmel	Rust and Powdery mildew	Urech *et al.*1979
14.	Tebuconazole	Folicur Raxil	Rust	Kaspers *et al.* 1987
15.	Triadimefon	Bayleton	Rust	Grewe and Buchel, 1973
16.	Triadimenol	Bayton Bafidan Summit	Powdery mildews	Anon., 1976
17.	Triazbutil	Indar	Powdery mildew, blast	Anon., 1970
18.	Tricyclazole	Beam	Blast	Froyd *et al.* 1976
19.	BAS-45406F	-	Powdery mildews	Pommer and Zech, 1983
20.	M-14360	-	Rust	Garavaglia *et al.*1988

21.	RH-7592	-	*Helminthosporium*	Driant *et al.* 1988

Source : Vyas (1993)

Table 2. Bioefficacy of hexaconazole against plant pathogens

S. No.	Crop	*Pathogen*	Reference
1.	Rice	*Rhizoctonia solani*	Chia Tiohuat (1997) Damodara Naidu and Venkata Rao (1997)
2.	Wheat	*Puccinia spp.* *Septoria spp.* *Erysiphe graminis tritici* *Pseudocercosporella herpotrichoides* *Fusarium spp.*	Waller *et al.* (1990)
3.	Wheat	*Septoria nodorum* *Mycosphaerella graminicola*	Eynard and Shephard (1990)
4.	Maize	*Helminthosporium maydis*	Thakore and Rajveersingh (1995)
5.	Pea	*Uromyces fabae*	Gupta and Shyam (1998)
6.	French bean	*Puccinia griseola*	Mathew *et al.* (1998)
7.	Groundnut	*Sclerotium rolfsii* *Rhizoctonia. solani*	Brown *et al.* (1989b)
8.	Groundnut	*Cercospora arachidicola*	Shephard *et al.* (1986)
9.	Chickpea	*Sclerotium rolfsii*	Tiwari (1995)
10.	Soybean	*Phakopsora pachyrhizi*	Senthil *et al.* (2000) Patil and Anahosur (1998)
11.	Sunflower	*Sclerotium rolfsii*	Tiwari (1995)
12.	Chilli	*Colletotrichum capsici*	Sharma *et al.* (1998)
13.	Bottle gourd	*Drechslera spp.* *Aspergillus spp*	Sharma *et al.* (1998)
14.	Cucurbits	*Sphaerotheca fuligena*	Torre *et al.* (1999)
15.	Banana	*Mycosphaerella musicola*	Chia Tiohuat (1997)
16.	Apple	*Venturia inaequalis*	Biggs and Warner (1987) Brown *et al.* (1989b) Shephard *et al.* (1986)

17.	Apple	*Nectria galligena*	Khosla and Gupta (1992) Cooke (1999)

(Contd...)

Table 2. Contd...

S. No.	Crop	*Pathogen*	Reference
18.	Apple	*Podosphaera leucotricha*	Shephard *et al.* (1986)
19.	Grape	*Phakopsora ampelopsidis*	Bhardwaj (1997)
20.	Grape	*Uncinula necator*	Shephard *et al.* (1986) Heaney *et al.* (1986) Varalakshmi *et al.* (2000)
21.	Grape	*Guignardia bidwelli* *Phyllosticta fragaricola*	Heaney *et al.* (1986)
22.	Cedar apple	*Gymnosporangium juniperi virginianae* *Taphrina deformans* *Coryneum beijerinckii*	Shephard *et al.* (1986)
23.	Cashew	*Oidium anacardii*	Topper *et al.* (1998)
24.	Coffee	*Hemileia vastatrix*	Chandra Mouli *et al.* (1997) Brown *et al.* (1989a) Shephard *et al*. (1986)
25.	Tea	*Exobasidium vexans*	Premkumar *et al.* (1998)
26.	Rose	*Phragmidium mucronatum* *Diplocarpon rosae*	Margina and Zheljazkov (1996)
27.	Cumin	*Alternaria burnsii*	Akbari *et al.* (1996)
28.	Zinnia	*Alternaria carthami*	Wu and Chou (1995)

CHAPTER III

MATERIALS AND METHODS

3.1. Plant material

Okra cultivar, Co-1 (susceptible to powdery mildew) (Plate 1 and 2) and chilli cultivar, T_4 (susceptible to powdery mildew) (Plate 3) were used throughout the study. The seed materials of okra and chilli were obtained from orchard, Department of Olericulture, Tamil Nadu Agricultural University, Coimbatore.

3.2. Bioefficacy of hexaconazole 5 SC - Field experiments

3.2.1. Management of okra powdery mildew

To test the efficacy of hexaconazole 5 SC against powdery mildew of okra, three field trials were conducted in which two trials at Allanthurai, Coimbatore during Oct-Dec, 1999 and Dec. 1999 to Feb. 2000 and one trial at Puthupalayam, Coimbatore during Oct-Dec, 2000. The trials were laid out in a randomized block design (RBD) with three replications by maintaining a plot size of 5.0 x 4.0 m^2 (Plate 4). The cultivar Co-1 was raised and the package of practices were followed as per the crop production guide (2000). Following were the treatments:

1. Hexaconazole 5 SC (Anvil ®) 500 ml ha^{-1} (25 g a.i. ha^{-1})
2. Hexaconazole 5 SC 750 ml ha^{-1} (37.5 g a.i. ha^{-1})
3. Hexaconazole 5 SC 1000 ml ha^{-1} (50 g a.i. ha^{-1})
4. Hexaconazole 5 SC 1500 ml ha^{-1} (75 g a.i. ha^{-1})
5. Mancozeb (Indofil M-45) 1 kg ha^{-1}

6. Propiconazole (Tilt) 1000ml ha^{-1}

7. Dinocap (Karathane) 1000ml ha^{-1}

8. Wettable sulphur 1500 ml ha^{-1}

9. Control (untreated)

Three sprays of each fungicide was given at 15 days interval from 45th day after planting. Powdery mildew disease incidence was recorded at 10th day after every spray on a randomly selected plants based on a 0-5 grade scale (Tajider Singh *et al*., 1994).

0 = No symptoms

1 = Small white specks on leaf covering 0.1% to 5% of leaf area

2 = 5.1 to 15% of leaf area affected

3 = 15.1 to 30% of leaf area affected

4 =30.1 to 50% of leaf area affected

5 = >50 per cent of the leaf area affected

The per cent disease index (PDI) was calculated as given by McKinney (1923).

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Number of plants observed}} \times \frac{100}{\text{Maximum disease grade}}$$

At the end of the season, fruit yield was recorded in each plot.

To study the persistence of hexaconazole, the fruit samples were collected from the hexaconazole treated plots before the spray and after the spray at zero day (one hour after spray), 1, 3, 5, 7 and 10 days after third spray, stored in 0.5 M methanolic sodium hydroxide and kept in deep freezer at –70 °C.

3.2.2. Management of chilli powdery mildew

To test the efficacy of hexaconazole 5 SC against powdery mildew disease of chilli, three field trials were conducted in which two trials at Thanneer panthal nearer to Mathampatti during Oct-Mar, 1999-2000; Aug-Jan, 2000-2001and one trial at Pudhupalayam, Coimbatore during Aug-Jan, 2001-2002 where epidemics of powdery mildew occur annually. The trials were laid out in a randomized block design (RBD) with three

replications by maintaining a plot size of 5.0 x 4.0 m^2 and the cultivar T4 was used (Plate 5). Package of practices were followed as per the crop production guide (2000). Following were the treatments:

1. Hexaconazole 5 SC (Anvil ®) 500 ml ha^{-1} (25 g a.i. ha^{-1})
2. Hexaconazole 5 SC 750 ml ha^{-1} (37.5 g a.i. ha^{-1})
3. Hexaconazole 5 SC 1000 ml ha^{-1} (50 g a.i. ha^{-1})
4. Hexaconazole 5 SC 1500 ml ha^{-1} (75 g a.i. ha^{-1})
5. Mancozeb (Indofil M-45) 1 kg ha^{-1}
6. Propiconazole (Tilt) 1000 ml ha^{-1}
7. Tridemorph (Calixin) 1000 ml ha^{-1}
8. Wettable sulphur 1500 ml ha^{-1}
9. Control (Untreated)

Three sprays of each fungicide were given at 15 days interval from 45th day after planting. The powdery mildew disease incidence was recorded at 10th day after every spray on randomly selected plants from each plot using a 0-5 scale (Tajider Singh *et al*., 1994).

Disease score	Description
0	*No symptoms*
1.	Small white specks on leaf covering 0.1 to 5 %
2.	5.1 to 15 % of leaf area affected.
3.	15.1 to 30 % of leaf area affected.
4.	30.1 to 50 % of leaf area affected.
5.	> 50 % of leaf area affected and defoliation occurs.

Disease incidence was recorded from 5 different randomly selected spots of the field by counting affected plants. Disease intensities of foliar diseases were recorded on 0-5 scale on 10 randomly selected plants of each plot.

The per cent disease index (PDI) was calculated as given by McKinney (1923).

At the time of harvest, the fruit yield was recorded. Fruit samples were collected before the spray and after the spray at zero day (one hour after the spray), 1, 3, 5, 7 and 10 days after third spray from the hexaconazole treated plots, stored in 0.5 M NaOH prepared in methanol and kept in deep freezer at –70°C.

3.3. Compatibility of hexaconazole 5 SC with mancozeb and monocrotophos for controlling okra and chilli pests and powdery mildew

The experimental plots were prepared with the specifications mentioned in section 3.2. for both the crops at Pudhupalayam, Coimbatore *viz*., Aug-Nov, 2001 and Aug-Feb, 2001 simultaneously.

The fungicide mancozeb @ 1kg ha^{-1} and the insecticide monocrotophos @ 1000 ml ha^{-1} were tested for compatibility with the four concentration of hexaconazole *viz*., 500, 750, 1000 and 1500 ml ha^{-1}. The pesticide mixtures were sprayed (prophyllactic) on both the crops and continued for 3 times at 15 days interval. Their efficacy against leaf hopper (*Amrasca devastans*) and whitefly (*Bemisia tabaci*) and powdery mildew (*Erysiphe cichoracearum*) in okra, thrips (*Scirtothrips dorsalis*), aphid (*Aphis gossypii*) and powdery mildew (*Leveillula taurica*) in chilli were recorded.

Incidence of powdery mildew in okra and chilli were recorded on 10 days after third spray by using the score charts mentioned earlier. The pest damage was also assessed at the same time by the following methods:

Okra:

1. Whitefly

The observations on whitefly damage were recorded on 10 randomly selected plants by counting the total number of leaves and the number of whiteflies per leaf. The mean population of whiteflies were calculated.

2. Leaf hopper

The number of hoppers in 3 leaves each of 10 plants per plot was counted

Chilli

1. Thrips

The thrips damage was recorded on 10 randomly selected plants 10 days after 3rd spray by counting total number of leaves. In that the population of thrips per leaf (scrapped leaves) and the mean population of thrips were calculated.

2. Aphid

The aphid damage was recorded on 10 randomly selected plants 10 days after 3rd spray by counting total number of leaves and infested leaves. The per cent aphid damage was calculated.

3.4. Phytotoxicity of hexaconazole 5 SC

Observations for the phytotoxicity effect of hexaconazole were made in the plants after each spray in all the field trials. All the leaves in the plants were regularly examined for injury to leaf tips and leaf surface, wilting, vein clearing, necrosis, epinasty and hyponasty. Leaf injury was graded based on visual rating on a 1-10 scale (CIB, 1989).

Grade	Per cent leaf injury
1	1-10
2	11-20
3	21-30
4	31-40
5	41-50
6	51-60
7	61-70
8	71-80
9	81-90
10	91-100

3.5. Persistence and harvest time residues of hexaconazole 5 SC

a. Sampling

Fruit samples were collected from all the four concentration of hexaconazole sprayed plots and control plot of okra and chilli from each trial before and after the spray at zero (one hour after spray), 1, 3, 5, 7 and 10 days after third spray. About 50 g of fruit samples from each replicate were collected and 25 g of working sample in duplicates were drawn by quartering method from the pooled sample. The working samples were stored in plastic containers with 100 ml of 0.5 M methanolic sodium hydroxide and stored at –70 °C in a deep freezer.

b. Extraction

Twenty five grams of samples were weighed and ground with 2 x 50 ml of 0.5 M methanolic sodium hydroxide. The ground materials were filtered through Buchner's funnel with Whatman No. 40 filter paper using the same solvent mixture. The filtrates were refluxed in a refluxion unit for one hour at 30°C. The refluxed filtrates were pooled and partitioned three times with 50 x 25 x 25 ml of hexane, in the presence of 20 ml of saturated sodium chloride and 50 ml of distilled water. The lower aqueous layer was

collected and pooled. It was again partitioned with 75 x 50 x 50 ml of dichloromethane. The dichloromethane extract was collected and pooled together and evaporated to near dryness.

c. Clean up

For column chromatography, 1.5 cm (dia) x 50 cm (length) glass columns were used. The drip tip of the column was plugged with cotton wool and packed to 6 cm height with activated silica gel sandwiched between 2 cm height of sodium sulphate on either side. The packed column was pre-wetted with dichloromethane. A small volume (5 ml) of dichloromethane was used to dissolve the residues adhering inside the beaker and allowed to pass through the packed column.

To elute the compound, 25 ml of 20 per cent acetone prepared in hexane was used. The eluate was concentrated to near dryness and the residue was dissolved in 5 ml of 20 per cent acetone prepared in hexane for final determination using Chemito 2865 Gas Liquid Chromatography (GC) fitted with Electron Capture Detector (ECD).

d. Gas Chromatography parameters

Column	:	Mixed column (1.5 % OV 17 + 1.95 % QF on gas chrom Q)
Oven temperature	:	220 °C
Injector temperature	:	250 °C
Detector temperature	:	280 °C
Volume injected	:	2 µl
Carrier gas	:	Nitrogen at 60 ml / min
Attenuation	:	8
Retention time	:	480 seconds

3.5.1. Recovery studies

a. Standards

The hexaconazole technical material obtained from M/s ICI – Syngenta Agrochemicals Ltd., Chennai, was used to prepare standards. One hundred mg of hexaconazole was transferred to a 100 ml volumetric flask, dissolved with acetone and the volume was made up. The flask was shaken well to get a homogenous stock solution of 1000 ppm and was stored in refrigerator.

The concentrated stock solution was brought to room temperature and one ml was transferred to a 100 ml volumetric flask, made up the volume and shaken well to obtain a homogenous solution of 10 ppm intermediate standard solution. From the intermediate stock solution, working standards of 0.5, 1, 2, 3 and 5 ppm were prepared and these standards were used in finding the retention time of the compound in GC.

b. Fortification

The samples were fortified at 0.5 ppm, 1 ppm, 3 ppm and 5 ppm level by adding the solution of working standards to fortify the fruits of okra, chilli and soil.

c. Quantification

The amount of residue was calculated by comparing the sample response with the response of standard by using the formula

$$\text{Residues in ppm} = \frac{Hs}{H\ std} \times \frac{Wstd}{Ws} \times \frac{Vex}{Vs} \times \frac{As}{A\ std} \times RF$$

Where,

Hs – Peak height of the sample

H std – Peak height of the standard

W std – Weight of the standard in ηg

Ws – Weight of the sample in g

Vex – Volume of the final extract in ml

Vs – Volume of the sample injected in μl

As – Attenuation of the sample

A std – Attenuation of the standard

RF – Recovery Factor

3.6. Green house studies

3.6.1. Inoculation of pathogens

a. *Erysiphe cichoracearum* DC

Conidia of *E. cichoracearum* were collected from the field at Mathampatti, Coimbatore and the leaves showing the powdery mildew symptoms were surface disinfected with $HgCl_2$ (0.1%). Rinsed in tap water and kept in moist chamber to induce sporulation. Conidial inoculum was prepared by scrapping the conidia from sporulating area and suspended in sterile distilled water. The spore concentration was determined with a haemocytometer and adjusted to give approximately 5×10^4 spores ml^{-1}. The conidial suspension was sprayed on plants raised in pot culture with an atomizer ensuring that both the surface of leaves were completely wetted. After the symptoms noticed, the crop was sprayed with the different treatments. First spraying was given at 45th day after sowing and subsequent sprays at 15 days interval for 2 times. The powdery mildew incidence was recorded after the last spray based on 0-5 scale.

b. *Leveillula taurica* (Lev.) Arn.

The chilli leaves showing typical powdery mildew symptoms were collected. The leaves were surface disinfected with $HgCl_2$ (0.1%). Rinsed in tap water and kept in moist chamber to induce sporulation. Conidial inoculum was prepared by scrapping the conidia from sporulating area and suspended in sterile distilled water. The spore concentration was determined with a haemocytometer and adjusted to give approximately 5×10^4 spores ml^{-1}. The conidial suspension was sprayed on plants raised in pot culture with an atomizer ensuring that both the surface of leaves were completely wetted. After the symptoms noticed, the crop was sprayed with the different treatments. First spraying was given at 45th day after sowing and subsequent sprays at 15 days interval for 2 times. The powdery mildew incidence was recorded after the last spray based on 0-5 scale (Tajider Singh *et al*., 1994).

3.6.2. Bioefficacy of hexaconazole 5 SC under green house condition

To determine the effect of different fungicides on okra and chilli powdery mildew, the experiments were conducted under green house condition (Plate 6 and 7).

The cultivar Co-1 okra seeds and the cultivar Co-1 chilli seeds were sown separately at the rate of 5 seeds/pot. Three replications were maintained in a randomised block design. The crop was sprayed with the treatments described in section 3.2. First spraying was given during the appearance of the symptoms for both the crops and the second spray at 15 days interval. Leaf samples were collected at 24 h and 72 h after last spray from each treatment for biochemical analysis.

Disease incidence was assessed based on 0-5 scale (Tajider Singh *et al*., 1994) for both the crops (Plate 8 and 9) at 10 days after last spraying. The PDI was calculated by using McKinney's formula.

3.7. Compatibility test with biocontrol agents – Poisoned food technique (Schmitz, 1930)

The biocontrol agents *viz*., *Trichoderma viride* and *T. harzianum*, were tested for their compatibility with hexaconazole. PDA was used as the basal medium to which calculated quantities of hexaconazole was separately mixed aseptically after sterilizing the medium to give required concentrations *viz*., 0.001, 0.01, 0.05, 0.1, 0.5 and 1.0 ppm. For each concentration, hexaconazole was measured into a 100 ml Ehrlenmeyer flask containing 100 ml of the sterilized and melted medium, mixed thoroughly by gently swirling the flask, poured in to a sterile Petri dish and allowed to solidify. A 9 mm actively growing PDA culture disc of the respective biocontrol agents were inoculated at the centre of the plate and the plates were incubated in the inverted position at room temperature (28 ± 2°C). Inoculated PDA medium with respective biocontrol agents and without hexaconazole served as control. Three replications were maintained for each concentration for each biocontrol agent. The radial growth of mycelium was measured periodically at 24 h interval. The per cent growth inhibition was calculated by the formula of Vincent (1927)

$$I = \frac{C - T}{C} \times 100$$

Where

I - Inhibition of mycelial growth

C - Mycelial growth in control

T – Mycelial growth in treatment

The same technique was used to test the efficacy of hexaconazole at different concentrations against *Colletotrichum capsici* and *Alternaria capsici*.

The bacterial biocontrol agents *viz*., *Pseudomonas fluorescens* Pf 1 and *Bacillus subtilis* were inoculated into King's B broth incorporated with different concentrations of hexaconazole. The O.D values were recorded at 595 nm for *P. fluorescens* and 659 nm for *B. subtilis*.

3.8. Effect of hexaconazole on spore germination

3.8.1. *Erysiphe cichoracearum* DC

Spore germination was conducted on detached leaves of okra, cultivar Co-1. The leaves were washed in distilled water and air dried. Hexaconazole at different concentrations (100, 500, 1000 and 2000 ppm) were placed individually on the surface of the leaves along with the checks *viz*., mancozeb (1000 ppm) and control (sterile water) and the droplets evenly spread with fine hair camel brush and allowed to air-dry (Dhurj *et al*., 1994). The treated leaves were inoculated with powdery mildew pathogen conidia. Three leaves from each treatment were transferred to a Petri dish with petiole dipped in water and incubated at 20°C. Each treatment was replicated three times. The leaves were observed at different day intervals (2nd, 3rd and 15th day) under microscope equipped with fine light arrangement and data on spore germination was recorded.

3.8.2. *Leveillula taurica* (Lev.) Arn.

Spore germination was conducted on detached leaves of chilli, cultivar Co-1. The leaves were washed in distilled water and air dried. Hexaconazole at different concentrations (100, 500, 1000 and 2000 ppm) were placed individually on the surface of the leaves along with the checks *viz*., mancozeb (1000 ppm) and control (sterile water) and the droplets evenly spread with fine hair camel brush and allowed to air- dry (Dhurj *et al*., 1994). The treated leaves were inoculated with powdery mildew pathogen conidia. Three leaves from each treatment were transferred to a Petri dish with petiole dipped in water and incubated at 20°C. Each treatment was replicated three times.

The leaves were observed at different day intervals (2nd, 3rd and 15th day) under microscope equipped with fine light arrangement and data on spore germination was recorded.

3.9. Effect of hexaconazole 5 SC on phylloplane and rhizosphere microorganisms

a. Phylloplane

The okra and chilli plants were raised in pot culture and sprayed with hexaconazole at different concentrations as mentioned in section 3.2.1. and 3.2.2. Leaf samples from each concentration and control plants were collected at zero, 1, 3 and 5 days after last spraying. One gram of leaf sample was weighed on each day and cut into small bits by means of a sterilized scalpel. The leaf bits were suspended in 10 ml of sterile distilled water, thoroughly shaken for five min and allowed to stand for five min to give 10^{-1} dilution.

b. Rhizosphere

The respective plants of okra and chilli from which the leaf samples collected were pulled out at different day intervals *viz.,* 0, 1, 3 and 5 days. The soil adhering the root surface area (rhizosphere) was collected. One gram of the rhizosphere soil was suspended in 10 ml of sterile distilled water, thoroughly shaken for five min and allowed to stand for five min to give 10^{-1} dilution.

3.9.1. Isolation and enumeration of phylloplane and rhizosphere microflora by serial dilution agar plating method

From 10^{-1}dilution, 1 ml aliquot was added to another 9 ml of sterile water blank to give 10^{-2} dilution. Likewise dilutions up to 10^{-5} were prepared. For bacteria, 15 ml of nutrient agar medium was added to plates containing 1 ml of 10^{-5} dilutions and for fungi 15 ml of PDA medium was added to plates with 1 ml of 10^{-3}dilution. The plates were

gently rotated for mixing the inoculum with the medium. Three replications were maintained for each concentration of hexaconazole treatment.

Upon solidification of the media, the plates were incubated at 25°C for 2 to 5 days. After the incubation period, the plates were observed for number of colonies of bacteria and fungi from respective dilution. The number of organisms per gram of leaf as well as rhizosphere soil were calculated using the following formula,

$$\text{Number of colony forming units / g} = \frac{\text{Number of colonies} \times \text{Dilution factor (average of 3 replicates)}}{\textbf{Fresh weight of leaf / rhizosphere soil}}$$

3.10. Induced Systemic Resistance

a. Samples

The leaf samples were collected from the plants raised in pot culture under green house condition. The okra plants were sprayed with different concentration as mentioned in section 3.2.1. The chilli plants were also sprayed with different concentrations as mentioned in section 3.2.2. The pathogens *E. cichoracearum* and *L. taurica* were inoculated on okra and chilli respectively on 2 days after fungicide spraying. The samples were collected on 0, 1, 3 and 5 days after inoculation. The samples were also collected on respective days from the fungicide sprayed, uninoculated plants for the assay of phenyl alanine ammonialyase activity. The samples were collected at 24 h and 72 h after spray for the assay of peroxidase as polyphenol oxidase activity.

b. Enzyme extract

About five hundred mg of leaf samples of okra and chilli were ground with 5 ml of 0.1M sodium phosphate buffer, pH 7.0. The ground materials were centrifuged at 10,000 rpm for 15 min. The supernatant was taken as enzyme source.

3.10.1. Assay of peroxidase (PO)

Assay of peroxidase activity was carried out as per the procedure described by Hammerschmidt *et al.* (1982). The reaction mixture consisted of 5.0 ml of 0.1M sodium phosphate buffer, pH 7.0, 0.05 ml of 0.25 per cent (w/v) guaiacol and 100 µl of enzyme extract. About five hundred microlitre of 0.1M hydrogen peroxide was added finally to initiate the reaction, which was followed colorimetrically at 470 ηm. The enzyme preparation added with 0.05 ml of 0.25 per cent guaiacol served as blank. Peroxidase activity was expressed as change in absorbance at 470 ηm min^{-1} g^{-1} fresh tissue.

3.10.2. Assay of polyphenol oxidase (PPO)

Assay of polyphenol oxidase activity was carried out as per the procedure described by Srivastava (1987). Exactly 2 ml of the extract and 3 ml of the phosphate buffer were pippetted into cuvettes and mixed by inverting it. The colorimeter was set at 495nm and the absorbance was adjusted to zero by placing the cuvette. The cuvette was removed and 1 ml of catechol was added in phosphate buffer and mixed again. The tube was placed immediately in the colorimeter and change in absorbance was recorded at every 30 sec up to 3 min. Control was also maintained without the substrate.

3.10.3. Assay of phenylalanine ammonialyase (PAL)

Assay of phenylalanine ammonialyase activity was carried as per the procedure given by Ross and Sederoff (1992). One gram of tissue was extracted in 3 ml of ice cold (4°C) 0.1M potassium phophate buffer (pH 8.0) containing 1.4 Mm 2- mercaptoethanol and 0.1 gm of insoluble PVP with the help of pestle and mortar. Filtered through Whatman No. 1 filter paper and centrifuged the filtrate at 15,000 g at 4°C for 20 min. The supernatant was used as a crude enzyme extract.

The reaction mixture was prepared for sample cuvette with 0.3 ml of crude enzyme extract, 1.2 ml of 0.1M borate buffer (pH 8.8) and 1.5 ml of 12 mM L- phenylalanine in a test tube. For reference cuvette (blank), the mixture 0.3 ml of crude enzyme extract, 2.7 ml of 0.1M borate buffer (pH 8.8) was prepared. After 30 min, the reaction was stopped by addition of 0.5 ml of 1M trichloro-acetic acid and incubated the assay mixture at 37°C for 5 min. The absorbance was measured at 290 nm and extinction coefficient of 9630 $m^{-1}cm^{-1}$ for trans-cinnamic acid in 0.1M borate buffer (pH 8.8) was used. The absorbance 9630 is equal to 1 mol / l / min. Otherwise the absorbance is 0.963, the product formed is 100 n. mol / ml / min. The enzyme activity was expressed on fresh weight basis (μ mol min^{-1} g^{-1} fresh weight) or as specific activity (μ katal mg^{-1} protein), one microkatal is defined as amount of enzyme that catalyses the formation of 1μ mol of product sec^{-1} or in units (1 unit = 1μ mol min^{-1}) or in katal (1katal = 1mol s^{-1}).

3.10.4. Native gel electrophoresis

Peroxidase isozymes

The enzyme extract prepared from chilli leaves by the above mentioned method was loaded. For native anionic polyacrylamide gel electrophoresis, resolving gel of eight

per cent acrylamide concentration and stacking gel of four per cent acrylamide concentration were prepared. Electrophoresis was carried out at constant voltage of 65 V in cold conditions. After electrophoresis, the gels were stained with the staining solution containing 0.05 per cent benzidine and 0.03 per cent hydrogen peroxide in 0.02 M acetate buffer (pH 4.5). After staining for three min, the gel was immersed in seven per cent acetic acid for 3 min washed with distilled water (Nadlony and Sequira, 1980).

Polyphenol oxidase isozymes

The enzyme extract prepared from chilli leaves by the above mentioned method was loaded. For native anionic polyacrylamide gel electrophoresis, resolving gel of eight per cent acrylamide concentration and stacking gel of four per cent acrylamide concentration were prepared. Electrophoresis was carried out at constant voltage of 65 V in cold conditions. After electrophoresis, the gels were stained for 30 min in 0.1 per cent p-phenylenediamine in 0.1M potassium phosphate buffer (pH 7.0) followed by 10mM catechol in the same buffer (Jayaraman, *et al*., 1987).

3.11. Biochemical changes

The okra and chilli plants were raised and sprayed with hexaconazole and other fungicides as mentioned in section 3.2.1. and 3.2.2. The samples were collected at 24 h and 72 h after last spraying. The leaves collected on respective time intervals were used for analysis of chlorophyll, protein and phenol contents.

3.11.1. Chlorophyll

Chlorophyll *a*, *b* and total chlorophyll contents were estimated by the procedure of Yoshida *et al.* (1971). About 500 mg of okra and chilli leaf samples were weighed and ground with 10 ml of acetone (80 %) in a pestle and mortar. It was centrifuged at 8000 rpm for 10 min. The supernatant solution was collected, made up to 25 ml using acetone

(80 %) and read at 645, 663 and 652 ηm for chlorophyll *a*, *b* and total chlorophyll contents respectively. The 80 per cent acetone alone was used as blank. Chlorophyll contents were expressed as mg g^{-1} of fresh weight.

$$\text{Chl } a = \frac{[12.7 \times \text{O.D at 663 ηm}] - [2.69 \times \text{O.D at 645 ηm}]}{1000 \times \text{weight of the sample}} \times \text{volume}$$

$$\text{Chl } b = \frac{[22.9 \times \text{O.D at 645 ηm}] - [4.68 \times \text{O.D at 663 ηm}]}{1000 \times \text{weight of the sample}} \times \text{volume}$$

$$\text{Total chlorophyll} = \frac{\text{O.D at 652 ηm} \times 1000 \times \text{volume}}{34.5 \times 1000 \times \text{weight of the sample}}$$

3.11.2. Soluble protein

Soluble protein content of okra and chilli leaves was estimated as per the method of Bradford (1976). About 500 mg of the sample was ground well with pestle and mortar in 5 ml of sodium phosphate buffer, pH 7.0. The contents were centrifuged at 8000 rpm for 10 min and the supernatant was used for protein estimation. One hundred microlitre of sample extract was taken in test tubes and made up to five hundred microlitre with sodium phosphate buffer. One ml of distilled water served as blank. Coomassie brilliant blue (2.5 ml) G-250, which was diluted @ 1:4 with distilled water was added to each tube including blank, mixed well and allowed to incubate the tubes at room temperature

for 5 min. After 5 min, the soluble protein absorbance was read immediately (within half an hour) at 595 ηm. The amount of protein was calculated from standard graph prepared with different concentrations of bovine serum albumin and expressed in terms of mg g^{-1} of fresh tissue.

3.11.3. Total phenol

Total phenol content was estimated following the procedure of Bray and Thorpe (1954). About 500 mg of leaf samples were extracted with 10 ml of 80 per cent ethanol. The ethanolic extract was boiled for 10 min in water bath. After cooling, the extract was centrifuged at 8000 rpm for 10 min. The volume of the supernatant was made up to 10 ml with 80 per cent ethanol. One hundred microlitre of ethanolic extract was evaporated in boiling water bath, added with 6 ml of water, 500 µl of Folin-ciocalteau reagent and kept for 10 min. After incubation, 2 ml of 20 per cent sodium carbonate solution was finally added and incubated for 30 min. The intensity of blue colour developed was measured at 660 ηm. The amount of phenol was calculated from the standard graph prepared with different concentrations of catechol. A blank containing all the reagents excluding the plant extract was used. The phenol content was expressed in catechol equivalents as mg g^{-1} fresh tissue.

3.12. Residues of hexaconazole 5 SC in harvested fruits and soil

3.12.1. Fruit samples

Samples of 25 g fruits of okra and chilli were collected from all the field trials mentioned in section 3.2 and used to determine harvest time residues by the method as described in section 3.5.

3.12.2. Soil samples

a. Sampling

The surface soil samples of 1 kg from all the four concentrations of hexaconazole sprayed and control plots of okra and chilli grown experimental fields were collected after harvesting. About 50 g of fruit samples from each replicate were collected and 25 g of working sample in duplicates were drawn by quartering method from the pooled sample. The working samples were kept at –70 °C in a deep freezer.

b. Extraction

Soil sample (50 g) was blended and placed in Soxhlet apparatus and ran for six hours in 300 ml of acetonitrile : water (50 : 50). The acetonitrile : water portion was collected and partitioned with 20 ml of saturated sodium chloride and 200 ml of dichloromethane (100+50+50 ml). The lower aqueous phase was collected, pooled and condensed to dryness.

c. Clean up

The column was packed as described in 3.5.c. The packed column was prewashed with dichloromethane + methanol (95 : 5). The dried samples were dissolved in 5 ml of dichloromethane and allowed to pass through the column. To elute the compound, 25 ml of dichloromethane : methanol (95 : 5) was used. The eluate was dried and dissolved in 5 ml of 20 per cent acetone prepared in hexane and analysed in GC using ECD (section 3.5.d.).

3.13. Statistical analysis

All the experiments were replicated atleast three times and repeated atleast once. The data were analysed statistically by using analysis of variance test followed by Duncan's Multiple Range Test (DMRT) for comparison of treatment means (Gomez and

Gomez, 1992). The degradation of residues were statistically analyzed by following the methods as described by Timme *et al*. (1986).

CHAPTER IV

EXPERIMENTAL RESULTS

Studies were carried out to determine the bioefficacy of hexaconazole 5 SC against okra and chilli powdery mildew disease, compatibility with insecticides and biocontrol agents, it's persistence and phytotoxic effects. Biochemical changes, induced enzymes, phylloplane and rhizosphere changes in plants due to the treatment of chemical and the residues in harvested produces of both the crops were also carried out. The results of the experiments conducted both *in vivo* and *in vitro* are given in this chapter.

FIELD INVESTIGATIONS

4.1. BIOEFFICACY OF HEXACONAZOLE 5 SC

4.1.1. Okra Powdery Mildew

Three field trials (as mentioned in Chapter III) were conducted to determine the bioefficacy of hexaconazole 5 SC against okra powdery mildew on the susceptible cultivar Co-1. Hexaconazole 5 SC at four different concentrations *viz.,* 500 ml, 750 ml, 1000 ml and 1500 ml ha^{-1} (25, 37.5, 50 and 75 g a.i. ha^{-1} respectively) were sprayed along with standard fungicides, mancozeb 1 Kg ha^{-1}, propiconazole 1000 ml ha^{-1}, dinocap 1000 ml ha^{-1} and wettable sulphur 1500 ml ha^{-1}. The per cent disease index was recorded before spray and after third spray (last spray). The results revealed that all the fungicides after three sprays were significantly effective against okra powdery mildew when compared to control (Table 3). The highest dose of hexaconazole 5 SC (1500 ml ha^{-1}) was found to be superior to other treatments in reducing the disease index to the level of 1.21 followed by the doses at 1000 ml ha^{-1} (2.61 PDI), 750 ml ha^{-1} (3.82 PDI) and at 500 ml ha^{-1} (7.54 PDI). The efficacy of hexaconazole 5 SC increased with increase in the

concentrations and there was 97.7 per cent decrease of disease incidence recorded at 1500 ml ha^{-1} of hexaconazole. The performance at the dose of 500 ml ha^{-1} was on par with mancozeb at 1 Kg ha^{-1} with 10.73 PDI. Among the treatments, wettable sulphur at 1500 ml ha^{-1} was the least effective against okra powdery mildew (15.36 PDI).

The other standard recommended fungicides *viz.,* propiconazole at 1000 ml and dinocap at 1000 ml ha^{-1} also showed significant effect up to 77.55 and 72.90 per cent decrease of disease incidence over the control. The rate of disease spread was found to be decreased in treated plots. Control plots recorded the PDI of 27.13 initially and then it increased up to 53.77 PDI.

The yield was also recorded in each treated plot and almost all the treatments showed similar effect and recorded significantly higher yield than control. The highest yield was recorded from hexaconazole at 1500 ml ha^{-1} (3865.30 Kg ha^{-1}) sprayed plots. Wettable sulphur treatment recorded the lowest yield of 3198.17 Kg ha^{-1} as that of control (3076.18 Kg ha^{-1}).

Similar results were also observed from the second (Table 4) and third (Table 5) field trials. In the second field trial, the lowest disease index was recorded in hexaconazole at 1500 ml ha^{-1} (1.27 PDI) (Plate 10) followed by other doses *viz.,* 1000 ml ha^{-1} (4.23 PDI), 750 ml ha^{-1} (6.58 PDI) and 500 ml ha^{-1} (8.87 PDI). All the treatments were significantly different from each other. Control plots recorded the maximum per cent disease index of 76.77 (Plate 11). The standard checks *viz.* mancozeb at 1 Kg ha^{-1} recorded the PDI of 9.70; propiconazole at 1000 ml ha^{-1} (13.33 PDI); dinocap at 1000 ml ha^{-1} (15.43 PDI) and wettable sulphur at 1500 ml ha^{-1} (18.27 PDI). The highest yield was recorded in hexaconazole at 1500

ml ha^{-1} (3603.17 Kg ha^{-1}) as in first trial followed by the same at 1000 ml ha^{-1} and significantly higher than the yield obtained from untreated plots (2859.17 Kg ha^{-1}).

In third field trial also, similar trend of results were obtained in which the higher dose of hexaconazole recorded the lowest PDI of 1.33 and maximum yield of 3782.03 Kg ha^{-1}. However the yield was on par with hexaconazole at 750 ml and 1000 ml ha^{-1} as well as mancozeb at 1 Kg ha^{-1}. Mancozeb at 1 Kg ha^{-1} recorded 8.73 PDI followed by propiconazole at 1000 ml ha^{-1} (10.47 PDI); dinocap at 1000 ml ha^{-1} (12.00 PDI) and wettable sulphur at 1500 ml ha^{-1} (14.87 PDI) as against the control (61.20 PDI). In all the field trials, the per cent yield increase was found to be ranging from 21 to 26 per cent (Table 5; Fig. 2). In the pooled analysis hexaconazole 1500 ml ha^{-1} recorded the least per cent disease index of 1.26 followed by the other concentrations *viz.,* 1000 ml (3.27), 750 ml (5.17) and 500 ml (7.61) compare to untreated control (63.91) (Table 5a).

4.1.2. Chilli powdery mildew

The bioefficacy of hexaconazole 5 SC was also tested against chilli powdery mildew under field conditions at four different doses *viz*., 500, 750, 1000 and 1500 ml ha^{-1}. Standard recommended fungicides such as mancozeb at 1 Kg ha^{-1}, propiconazole at 1000 ml ha^{-1}, tridemorph at 1000 ml ha^{-1} and wettable sulphur at 1500 ml ha^{-1} were treated as checks. Observations were made before the fungicide spray and after the last spray (3rd spray) and the results are given in Table 6, 7 and 8.

In the first field trial it was opined that all the fungicides were effective against chilli powdery mildew disease and were significantly superior than the control. The lowest per cent disease index was observed in hexaconazole at 1500 ml ha^{-1} sprayed plots

(1.60 PDI) followed by the respective doses of 1000 ml ha^{-1} (3.30 PDI); 750 ml ha^{-1} (4.00 PDI) and 500 ml ha^{-1} (5.90 PDI). The efficacy at 500 ml ha^{-1} was statistically similar to the effect of mancozeb 1 Kg ha^{-1} (7.30 PDI). The efficacy of hexaconazole 5 SC against powdery mildew increased with increase in concentration. Among the fungicides tested, wettable sulphur at 1500 ml ha^{-1} showed the least performance recorded 11.00 PDI. The standard checks *viz.,* propiconazole at 1000 ml ha^{-1} showed 9.00 PDI and tridemorph at 1000 ml ha^{-1} showed 9.80 PDI (Table 6).

Hexaconazole at 1500 ml ha^{-1} recorded the highest yield of 7674.73 Kg ha^{-1} as compared to 6809.10 Kg ha^{-1} in control. Significant yield increase was found among the different doses of hexaconazole treated plots. Among other fungicide treated plots, wettable sulphur at 1500 ml ha^{-1} recorded the lowest yield of 7047.33 Kg ha^{-1}.

The same trend of results were also observed from second (Table 7) and third (Table 8) field trials. The highest dose of hexaconazole at 1500 ml ha^{-1} showed the least per cent disease index of 2.03 followed by the respective doses *viz.,* 1000, 750 and 500 ml ha^{-1} (4.20 PDI, 5.83 PDI and 7.03 PDI respectively) in the second field trial. The yield was also found to be maximum with 7782.50 Kg ha^{-1} in hexaconazole 1500 ml ha^{-1} treated plots. Control plots recorded the maximum PDI of 63.3 and the lowest yield of 6712.53 Kg ha^{-1} (Table 7).

Similarly in third field trial also the hexaconazole at 1500 ml ha^{-1} was found to be the best among the doses of hexaconazole and recording the lowest PDI of 1.73 with the maximum yield of 7416.73 Kg ha^{-1} (Plate 12). Mancozeb at 1 Kg ha^{-1} was statistically on par with hexaconazole 500 ml ha^{-1} both in disease reduction and yield. Standard checks *viz.,* propiconazole at 1000 ml ha^{-1} and tridemorph at 1000 ml ha^{-1} recorded the PDI of 7.80; and 9.30 respectively and wettable sulphur at 1500 ml ha^{-1} showed 12.10 PDI. Control plot recorded the maximum PDI (69.17) with the lowest yield of 6240.33 Kg ha^{-1} (Table 8; Plate 13). The pooled analysis revealed that hexaconazole 1500 ml ha^{-1} showed the least per cent disease index of 1.79 followed by the other concentration *viz.,* 1000 ml (3.70), 750 ml (4.64) and 500 ml (5.94) as against the control recorded 63.02 PDI (Table 8a).

4.2. Compatibility of hexaconazole 5 SC with mancozeb and monocrotophos

4.2.1. Okra

Compatibility of hexaconazole 5 SC at 500 ml ha^{-1} with mancozeb at 1 Kg ha^{-1} and monocrotophos at 1000ml ha^{-1} and their combined efficacy was studied against pests and powdery mildew disease of both okra and chilli in field conditions. The observations were taken 15 days after last spray (third spray). (Table 9 and 10).

The combination treatment of hexaconazole at 500 ml ha^{-1} with monocrotophos at 1000 ml ha^{-1} showed the least population of whitefly (8.03) and leafhopper (11.33) followed by monocrotophos alone at 1000 ml ha^{-1} recorded 9.67 and 14.24 respectively. The efficacy of monocrotophos at 1000 ml ha^{-1} alone and combined with the hexaconazole at 500 ml ha^{-1} were on par with each other. This was followed by the combination treatment of hexaconazole at 500 ml ha^{-1} + mancozeb at 1 Kg ha^{-1} which showed the whitefly population of 24.47 and leaf hopper population of 43.41. The treatment with mancozeb 1 Kg ha^{-1} was on par with hexaconazole treatment at 500 ml ha^{-1} recording the whitefly population of 27.26 and 30.70 respectively and the leaf hopper population of 48.53 and 49.23 respectively as against control with the whitefly population of 35.53 and leaf hopper population of 63.67. The combination treatment was good in reducing the pest incidence compared to individual insecticide application with regard to okra crop.

In case of powdery mildew incidence, the combination treatment of hexaconazole (500 ml ha^{-1}) with mancozeb (1 Kg ha^{-1}) recorded the least per cent disease index of 6.50 as against control (47.93) followed by mancozeb alone (10.10 PDI). The combination treatment of hexaconazole 500 ml ha^{-1} + monocrotophos 1000 ml ha^{-1} was found to be next best treatment in reducing the powdery mildew incidence of 11.70 PDI followed by hexaconazole alone (19.06 PDI). However the combination treatment was found to be the best when compared to the individual application in reducing both the pests and powdery mildew disease of okra (Table 9; Fig.4).

4.2.2. Chilli

In chilli crop, the combination treatment of hexaconazole at 500 ml ha^{-1} with monocrotophos at 1000 ml ha^{-1} recorded the least thrips population of 8.73 and 16.63 per cent aphid damage followed by the treatment with monocrotophos alone showed 15.70 in number and 19.25 per cent respectively. The combination treatment of hexaconazole (500 ml ha^{-1}) plus mancozeb (1 Kg ha^{-1}) showed the thrips population of 26.87 and aphid damage of 23.98 per cent followed by mancozeb alone showed thrips population of 28.80 and aphid damage of 28.82 per cent and these two treatments were statistically on par with each other. Control plots showed the thrips population of 42.13 and 69.73 per cent aphid damage. The combination treatment was found to be effective when compared to individual application.

The combination treatment of hexaconazole (500 ml ha^{-1}) plus mancozeb (1 Kg ha^{-1}) recorded the least PDI of 7.60 followed by mancozeb alone (11.20 PDI) as against control (52.53 PDI). Hexaconazole (500 ml ha^{-1}) plus monocrotophos (1000 ml ha^{-1}) also reduced powdery mildew disease incidence of 15.50 PDI (Table 10; Fig.5).

4.3. Persistence and dissipation of hexaconazole 5 SC

Hexaconazole 5 SC at four different doses *viz.* 500, 750, 1000 and 1500 ml ha^{-1} was determined for its persistence in okra and chilli crop. The samples collected at different intervals *viz.,* 0, 1, 3, 5, 7 and 10 days after spray were analyzed by using Gas Chromatography. The fortification studies were carried out to standardize the methodology which recorded 83.40 per cent recovery in okra crop and 83.51 per cent recovery of hexaconazole residues in chilli crop.

4.3.1. Okra

In the first field trial, maximum amount of hexaconazole residue was recorded at 0th day after last spray (0.46 µg g^{-1}) at 500 ml ha^{-1} and the minimum residue was observed on seventh day after spray (0.07 µg g^{-1}) at 750 ml ha^{-1} concentration whereas the highest concentration of hexaconazole at 1500 ml ha^{-1} recorded the maximum amount of residues of 1.29 µg g^{-1} on 0th day after last spray and the minimum of 0.15 µg g^{-1} was observed on seventh day after last spray (Table 11). Irrespective of all the trials, there was an increase in the initial deposits of hexaconazole with the increase in the doses (Fig.6).

With regard to second trial, the maximum amount of residues recorded at zero day after last spray was 0.43 µg g^{-1} at 500 ml ha^{-1} and the minimum amount of residue of 0.17 µg g^{-1} was recorded on tenth day after last spray. However the amount of residues was increased up to 0.65 µg g^{-1} at 1500 ml ha^{-1} at zero day after spray and the minimum was up to 0.25 µg g^{-1} on tenth day after spray. The amount of residues increased with increase in concentration (Table 11).

In third field trial also (Table 11), the same trend was observed as in second trial. Initially the amount of residues was high up to 0.32 µg g^{-1} on zero day after spray at 500 ml ha^{-1} and increased up to 0.38 µg g^{-1} at 1500 ml ha^{-1} and the minimum amount of residue was observed on tenth day up to 0.16 µg g^{-1} at 500 ml ha^{-1} and this was increased up to 0.24 µg g^{-1} at 1500 ml ha^{-1}. Among the three trials, the amount of residue or the persistence of hexaconazole was observed up to tenth day in second and third field trial whereas it was observed only up to seventh day after last spray in the first trial of okra. Unsprayed fruit samples were also analysed for any persistence of hexaconazole and the results depicted that there was no residue in untreated fruit samples of okra from zero day to tenth day intervals. In all the field trials, maximum amount of residues was observed in the highest concentration of hexaconazole treated plots and the minimum was observed in the lowest concentration.

The hexaconazole residues were declined/dissipated steadily during the subsequent five day intervals with progress in time in okra (Table 12; Fig.6). The residues were dissipated from 32.23 per cent to 96.52 per cent in first trial in okra, 9.66 per cent to 61.32 per cent and 13.43 per cent to 43.59 per cent in second, third trial respectively (Table 12). However, the slower rate of dissipation of hexaconazole residues in okra during second and third trial may be due to temperature difference.

The decline behaviour of sloughable hexaconazole residues was computed following seven transformation and the best fit was selected among them. Based on the regression co-efficient (r) of determination, the best fit observed in okra was first order kinetics for the hexaconazole in the first trial and it was second order, root function first order kinetics for the second, third trials respectively (Table 13).

The various statistical parameters like intercept (a), slope (b) of regression line and half life ($T_{0.5}$) with their confidence limits for the best fit function in okra are presented in Table 14. The first order kinetics for the dissipation of hexaconazole residues in okra recorded the half life ($T_{0.5}$) of 2.1160 to 2.9816 days (first trial) while 1.5^{th} order, second order, root function first order kinetics with the half-life ($T_{0.5}$) of 1.7868 to 5.8298 days, 5.8923 to 14.6155 days, 8.8906 to 22.7652 days respectively (Table 14).

The safe waiting period (T_{tol}) of the treated okra compared with the established tolerance limit of hexaconazole 0.05 ppm per Kg was worked out to be 11.17 to 15.47 days (Table 14).

4.3.2. Chilli

In the first field trial, among all the four treatments, the hexaconazole residues persisted up to seven days and then degraded to below detectable level (BDL). The maximum amount of residue was recorded at zero day after spray at 500 ml ha^{-1} (0.18 µg g^{-1}) and the minimum was observed on seventh day after spray (0.03 µg g^{-1}) whereas hexaconazole at 1500 ml ha^{-1} recorded the maximum residue of 0.43 µg g^{-1} on zero day after spray and the minimum was 0.13 µg g^{-1} on seventh day after spray. Irrespective of the trials, there was an increase in the initial deposits of hexaconazole with the increase in the doses (Table 15; Fig.7).

In the second trial, the initial amount of residue was 0.05 µg g^{-1} on 0th day and it was degraded to below detectable level (BDL) on third day itself at the lowest concentration of 500 ml ha^{-1}. However the maximum amount of residue of 0.32 µg g^{-1} recorded on 0th day and the minimum was 0.07 µg g^{-1} on seventh day after last spray at higher concentration of 1500 ml ha^{-1}. In this trial maximum amount of residues persisted up to seven days in chilli fruits when hexaconazole treated at higher concentration of 1500 ml ha^{-1} and 1000 ml ha^{-1} whereas the lowest concentration of 500 ml ha^{-1} and 750 ml ha^{-1} recorded the persistence of residues only up to three days after last spray (Table 15; Fig. 7)

In the third trial, the trend was different. The hexaconazole residue persisted only up to three days after spray. From fifth day onwards, it was found to be below detectable level (BDL). The initial amount of maximum residue was 0.04 µg g^{-1} at 0th day at 500 ml ha^{-1} and it was degraded to below detectable level (BDL) for subsequent day intervals. However the maximum residue was 0.37 µg g^{-1} on 0th day after spray and the minimum

was observed on third day recording 0.17 µg g^{-1} at high concentration of 1500 ml ha^{-1}. Among all the three trials, the persistence of hexaconazole was recorded up to seven days in the first and second trial whereas in the third trial, the persistence was recorded only up to third day after spray. The amount of residues increased with increase in doses.

In chilli, the residues dissipated from 36.94 per cent to 92.45 per cent in first trial, 36.50 per cent to 82.13 per cent and 27.02 per cent to 75.59 per cent in second, third trial respectively (Table 16). The best fit observed in chilli was first order kinetics in all the three trials and also followed the second order, root function first, second order kinetics (Table 17).

Various statistical parameters like intercept (a), slope (b) of regression line and half life ($T_{0.5}$) with their confidence limits for the best fit function in chilli are presented in Table 18. In chilli, the first order kinetics for the dissipation of hexaconazole residues recorded the half life ($T_{0.5}$) of 1.90 to 3.10 days while the second order showed the half life ($T_{0.5}$) of 2.04 days, root function first order, root function second order kinetics with the half life of 1.54, 1.23 days respectively (Table 18).

The safe waiting period (T_{tol}) of the treated chili compared with the established tolerance limit of hexaconazole 0.05 ppm per Kg was worked out to be 7.13 to 11.38 days (Table 18).

4.4. Phytotoxicity of hexaconazole 5 SC

The phytotoxicity of hexaconazole 5 SC at four different concentrations in all the three field trials of both okra and chilli crop was determined based on the parameters (as mentioned in Chapter III) and the observations recorded are given in Table 19 and 20.

Hexaconazole at highest dose of 1500 ml ha^{-1} showed leaf injury or phytotoxicity as vein clearing symptoms in okra crop in second trial only *i.e.* less than one per cent level based on the parameters (Table 19). There was no phytotoxicity of hexaconazole observed in all the other field trials of both okra and chilli crop.

4.5. Greenhouse experiments

4.5.1. Bioefficacy of hexaconazole against okra powdery mildew

In greenhouse studies, three trials were conducted to study the bioefficacy of hexaconazole 5 SC against okra and chilli powdery mildew disease. Hexaconazole at high concentration of 1500 ml ha^{-1} recorded the least per cent disease index of 19.73, 22.57 and 20.40 in first, second and third trials respectively (Plate 14) followed by the other concentrations *viz.,*1000 ml, 750 ml and 500 ml ha^{-1} which recorded 26.07, 29.47, 26.52 PDI; 31.07, 34.37 and 31.93 PDI; 36.10, 40.23 and 36.20 PDI respectively as against control (sterile water sprayed) recorded 87.27, 90.63 and 92.30 PDI in first, second and third trials respectively (Plate 15). However, all the fungicides were effective

against okra powdery mildew and were significantly different as against control. In all the three trials, wettable sulphur at 1500 ml ha^{-1} was least effective when compared to other fungicides (Table 21).

4.5.2. Bioefficacy of hexaconazole against chilli powdery mildew

Similar trend of results were observed in all the three trials of chilli crop. The hexaconazole 5 SC at 1500 ml ha^{-1} recorded the least per cent disease index of 20.60, 16.70 and 18.58 PDI respectively in first, second and third trials (Plate 16) followed by the other respective doses *viz.,* 1000 ml ha^{-1} (27.50, 25.53 and 25.80 PDI), 750 ml ha^{-1} (34.33, 31.43 and 32.24 PDI) and 500 ml ha^{-1} (38.27, 34.67 and 35.45 PDI) in the respective three trials as against control (sterile water sprayed) recorded 90.33, 93.33 and 94.60 PDI respectively. All the treated fungicides were found to be significantly effective against the disease however, wettable sulphur (1500 ml ha^{-1}) recorded maximum per cent disease index of 47.13, 44.70 and 43.18 PDI in first, second and third trials respectively (Table 22).

4.6. Spore germination (*in vitro)*

4.6.1. Spore germination of *Erysiphe cichoracearum* in different concentrations of hexaconazole 5 SC

The efficacy of different concentrations of hexaconazole (100, 500, 1000 and 2000 ppm) was tested against the spore germination of *Erysiphe cichoracearum* at different day intervals *viz.,* 2, 3 and 15 days after treatment and the observations were recorded and given in Table 23; Fig.8. The results showed that among the different concentrations of hexaconazole 5 SC, hexaconazole at 2000 ppm showed the least per cent spore germination of 14.93, 8.23 and 3.03 respectively on 2, 3 and 15 days after treatment followed by the other concentrations *viz.,* 1000 ppm (26.87%, 21.57% and 7.10%); 500 ppm (40.80%, 35.93% and 12.20%) and 100 ppm (51.77%, 44.17% and 20.27%) on 2, 3 and 15 days after treatment respectively as against control (sterile water) which recorded 78.66, 88.33 and 96.33 per cent respectively on 2, 3 and 15 days after treatment. Mancozeb at 1000 ppm treated as a check which recorded the per cent spore germination of 56.73, 50.33 and 23.30 respectively on 2, 3 and 15 days after treatment. From this

spore germination study, it is clear that immediately after treatment with hexaconazole at different concentrations, the per cent spore germination was maximum and it was reduced on 15 days after treatment of hexaconazole 5 SC. Also hexaconazole at higher dose of 2000 ppm recorded the least per cent spore germination.

4.6.2. Spore germination of *Leveillula taurica* in different concentrations of hexaconazole 5 SC

Hexaconazole at four different concentrations (as mentioned in 4.6.1.) were tested against the spore germination of *Leveillula taurica* at three different day intervals *viz.,* 2, 3 and 15 days after treatment. Mancozeb was used as a check. The hexaconazole at 2000 ppm recorded the least per cent spore germination of *Leveillula taurica viz.,* 15.43, 6.10 and 2.00 respectively at three different day intervals *viz.,* 2, 3 and 15 days after treatment followed by the other concentrations *viz.,* 1000 ppm (19.53%, 11.23% and 5.00%); 500 ppm (25.70%, 16.33% and 9.00%) and 100 ppm (31.90%, 23.47% and 12.00%) respectively on 2, 3 and 15 days after treatment as against control (sterile water) which recorded 71.00, 81.67 and 86.00 per cent spore germination respectively on 2, 3 and 15 days after treatment. However mancozeb at 1000 ppm recorded the per cent spore germination of 33.97, 25.50 and 17.00 on 2, 3 and 15 days after treatment respectively (Table 24; Fig.9).

4.7. Biochemical changes in okra and chilli crop induced by hexaconazole 5 SC

4.7.1. Chlorophyll content

Chlorophyll content of okra leaves was determined by colorimetric method at two different time intervals *viz.,* 24 h and 72 h after spray in hexaconazole treated and other standard fungicides treated plants and these were compared to control plants (untreated).

Among the different concentrations of hexaconazole, the highest concentration of hexaconazole (1500 ml ha^{-1}) recorded the highest chlorophyll content of *a, b* and total chlorophyll at 24 h and 72 h after spray followed by the other concentrations compare to untreated control plants.

Chlorophyll *a, b* and total chlorophyll content was found to be increased from the high concentration of hexaconazole (1500 ml ha^{-1}) to low concentration (500ml ha^{-1}) which recorded the chlorophyll *a* content of 1.17, 1.12, 1.04 and 1.01 mg g^{-1} (24 h after spray) and 1.55, 1.41, 1.26 and 1.15 mg g^{-1} (72 h after spray); chlorophyll *b* content of 0.58, 0.49, 0.47 and 0.40 mg g^{-1} (24 h after spray) and 0.89, 0.73, 0.68 and 0.51 mg g^{-1} (72 h after spray); total chlorophyll content of 1.74, 1.59, 1.46 and 1.40 mg g^{-1} (24 h after spray) and 2.15, 1.92, 1.75 and 1.52 mg g^{-1} (72 h after spray) as against control (untreated plants) which recorded the chlorophyll *a* content of 0.65 mg g^{-1}, chlorophyll *b* content of 0.24 mg g^{-1} and total chlorophyll content of 0.95 mg g^{-1} (24 h after spray) and 0.81, 0.31 and 1.10 mg g^{-1} content of chlorophyll *a, b* and total chlorophyll respectively (72 h after spray). The standard fungicides also showed significant chlorophyll content of okra leaves at the time interval of 24 h and 72 h after spray.

Hexaconazole at 1500 ml and 1000 ml ha^{-1} was on par with each other. Also the standard fungicides *viz.,* mancozeb, propiconazole, dinocap and wettable sulphur were on par with each other. The chlorophyll content increased in hexaconazole sprayed okra plants when compared to untreated control plants. The maximum chlorophyll content was recorded at 72 h after spray when compare to 24 h after spray especially when the plants were treated with high concentration of hexaconazole (1500 ml ha^{-1}) (Table 25).

Similarly in chilli crop also, the same trend was observed. Hexaconazole at high concentration (1500 ml ha^{-1}) showed the maximum chlorophyll *a, b* and total chlorophyll content *viz.,* 1.83, 0.74 and 2.28 mg g^{-1} (24 h after spray) and 1.93, 0.97 and 2.43 mg g^{-1} (72 h after spray) followed by other concentrations *viz.,* 1000 ml ha^{-1} recorded 1.68, 0.61 and 2.12 mg g^{-1} (24 h after spray) and 1.84, 0.78 and 2.35 mg g^{-1} (72 h after spray); 750 ml ha^{-1} recorded 1.54, 0.52 and 2.05 mg g^{-1} (24 h after spray) and 1.72, 0.64 and 2.28 mg g^{-1} (72 h after spray) and 500 ml ha^{-1} showed 1.42, 0.45 and 1.90 mg g^{-1} (24 h after spray) and 1.60, 0.51 and 2.10 mg g^{-1} (72 h after spray) as against control/untreated plants recorded 0.87, 0.20 and 1.33 mg g^{-1} (24 h after spray) and 1.08, 0.29 and 1.49 mg g^{-1} (72 h after spray) of chlorophyll *a, b* and total chlorophyll content respectively. All the standard fungicides recorded the significant increase of chlorophyll content at different time intervals. Similarly the increased rate of chlorophyll content was observed at 72 h after spray when compared to 24 h after spray (Table 26).

4.7.2. Total phenol content

The increase in phenol content was observed in all the hexaconazole sprayed plants (Table 27; Fig.10). Maximum phenol content was observed in hexaconazole at 1500 ml ha^{-1} (540.00 mg 100 g^{-1}) at 24 h after treatment and 740.00 mg 100 g^{-1} at 72 h after treatment followed by 1000 ml ha^{-1} (406.67 mg 100 g^{-1} and 613.30 mg 100 g^{-1}) at 24 and 72 h after treatment; 750 ml ha^{-1} (386.60 mg 100 g^{-1} and 510.00 mg 100 g^{-1}) and 500 ml ha^{-1} (343.33 mg 100 g^{-1} and 436.67 mg 100 g^{-1}) at 24 and 72 h after treatment as against unsprayed control recorded 273.32 mg 100 g^{-1} and 310.00 mg 100 g^{-1} at 24 h and 72 h after treatment. However hexaconazole at 500 ml ha^{-1} and mancozeb 1 Kg ha^{-1} treatments were on par with each other at 24 h after treatment (343.33 mg 100 g^{-1} and 366.69 mg 100 g^{-1} respectively). Standard fungicides *viz.*, propiconazole 1000 ml ha^{-1}, dinocap 1000 ml ha^{-1} and wettable sulphur 1500 ml ha^{-1} recorded the significant increase in phenol content of

340.00, 330.00 and 310.00 mg 100 g^{-1} at 24 h after spray and 386.68, 376.60 and 346.65 mg 100 g^{-1} at 72 h after treatment. All the treated fungicides showed significant increase in phenol content as against control unsprayed plants.

Similarly in chilli crop, phenol content was found to be maximum in hexaconazole sprayed at 1500 ml ha^{-1} recorded 813.30 mg 100 g^{-1} at 24 h after treatment and 850.00 mg 100 g^{-1} at 72 h after treatment followed by 1000 ml ha^{-1} (746.68 and 763.32 mg 100 g^{-1}); 750 ml ha^{-1} (560.00 and 671.70 mg 100 g^{-1}) and 500 ml ha^{-1} (520.00 and 620.00 mg 100 g^{-1}) respectively at 24 h and 72 h after treatment as against unsprayed control plants showed 410.00 and 383.41 mg 100 g^{-1} at 24 h and 72 h after treatment. Standard fungicides showed significant increase in phenol content however mancozeb (1 Kg ha^{-1}) and propiconazole (1000 ml ha^{-1}) treatments were on par with hexaconazole at 750 and 500 ml ha^{-1} at 72 h after treatment. The increase in phenol content was observed in standard fungicides *viz.,* mancozeb 1 Kg ha^{-1} (646.69 mg 100 g^{-1}) and propiconazole at 1000 ml ha^{-1} (620.00 mg 100 g^{-1}) and this was found to be higher when compared to hexaconazole at 750 ml ha^{-1} (560.00 mg 100 g^{-1}) and 500 ml ha^{-1} (520.00 mg 100 g^{-1}) at 24 h after treatment (Table 28; Fig.11). Total phenol content was found to be maximum at 72 h after treatment when compared to initial 24 h after treatment.

4.7.3. Soluble protein content

Significant increase in soluble protein content was observed in hexaconazole sprayed okra plants (at different concentrations) as compared to unsprayed control plants. Maximum soluble protein content was recorded in hexaconazole at 1500 ml ha^{-1} at two different time intervals *viz.,* 24 h after spray (604.00 mg 100 g^{-1}) and 72 h after spray (613.40 mg 100 g^{-1}) followed by other doses *viz.*, 1000 ml ha^{-1} (571.67 and 583.31 mg 100 g^{-1}); 750 ml ha^{-1} (560.00 and 568.33 mg 100 g^{-1}) and 500 ml ha^{-1} (550.00 and 556.67 mg 100 g^{-1}) at 24 h and 72 h after treatment respectively as against control recorded 480.00 and 433.28 mg 100 g^{-1} respectively at

24 h and 72 h after spray. Standard recommended fungicides *viz.,* mancozeb (1 Kg ha^{-1}), propiconazole (1000 ml ha^{-1}), dinocap (1000 ml ha^{-1}) and wettable sulphur (1500 ml ha^{-1}) recorded the significant increase in protein content. All the treated fungicides showed respective increase in protein content as against untreated control. Soluble protein content was maximum at 72 h after treatment when compared to 24 h after treatment (Table 27; Fig.10).

With regard to chilli crop, the maximum protein content was recorded in hexaconazole at 1500 ml ha^{-1} (545.00 and 628.30 mg 100 g^{-1}) at 24 h and 72 h after treatment respectively followed by the respective increase in doses *viz.,* 1000 ml ha^{-1} (506.70, 555.00 mg 100 g^{-1}); 750 ml ha^{-1} (443.50, 491.68 mg 100 g^{-1}) and 500 ml ha^{-1} (433.30, 461.72 mg 100 g^{-1}) respectively at 24 h and 72 h after treatment as against control untreated plants which recorded 374.20 and 370.00 mg 100 g^{-1} at 24 h and 72 h after spray respectively. Standard fungicides also recorded the significant increase in protein content. There was significant increase in all the treated fungicides and soluble protein content was maximum at 72 h after spray when compared to 24 h after spray (Table 28; Fig.11).

4.8. Molecular analysis of okra and chilli plants with ISR

4.8.1. Activity of phenylalanine ammonia lyase (PAL) treated with hexaconazole (with and without powdery mildew pathogen inoculation)

Induction of PAL activity was observed in okra plants due to the application of hexaconazole against *E. cichoracearum*. The activity of PAL was high in inoculated okra plants compared to uninoculated control. Results in Table 29 clearly revealed maximum PAL activity on 5th day in case of hexaconazole 1500 ml ha^{-1} application compared to other treatments against powdery mildew. Eventhough significant increase in PAL activity was observed in standard fungicidess *viz.,* mancozeb propiconazole, dinocap and

wettable sulphur, they were not upto the level as hexaconazole against the powdery mildew in okra plants.

In case of chilli similar results were obtained against the *Leveillula taurica*. Significant increase in PAL activity was observed in hexaconazole 1500 ml ha^{-1} treatment on 5th day compared to other standard fungicides against the powdery mildew. The data presented in table 30 showed that hexaconazole 5SC at gradual increase in higher concentrations increased the PAL activity compared to other treatments. Significant difference in PAL activity was observed between treatments, day interval and pathogen inoculation (Table 30).

4.8.2. Peroxidase (PO) activity in okra and chilli plants

It was inferred that application of hexaconazole 1500 ml ha^{-1} led to increase in PO activity against the powdery mildew pathogen. Gradual increase in PO activity was observed upto 72 h in okra plants compared to other treatments. There was no appreciable change in PO activity in standard fungicide and control plants (Table 31; Fig.12). Hexaconazole 1500 ml ha^{-1} treatment in chilli plants showed enhanced PO activity in the leaf against the powdery mildew pathogen. The data in table 32 clearly revealed that hexaconazole 5SC at the rate of 500, 750, 1000 and 1500 ml ha^{-1} showed gradual increase in PO activity upto 72h compared to standard fungicides and untreated control chilli plants (Fig.13). There was no significant difference in PO activity between standard fungicide treatments and control treatments in chilli plants .

4.8.2.1. Peroxidase isoforms

Native gel electrophoresis of hexaconazole treated, mancozeb treated and control chilli plants inoculated with powdery mildew pathogen showed different isoforms of PO. The different concentrations of hexaconazole *viz*. 1500 ml ha^{-1}, 1000 ml ha^{-1}, 750 ml ha^{-1} and 500 ml ha^{-1} showed induction of different thick isoforms of PO compared to mancozeb and control plants. Very weak and mild induction of PO isozymes was observed in case of mancozeb and control plants (Plate 17).

4.8.3. Polyphenol oxidase (PPO) activity in okra and chilli plants

Application of hexaconazole at different concentrations led to increase in PPO activity upto 72 h compared to other standard fungicides and untreated control okra plants against powdery mildew pathogen. Table 31 clearly revealed the induction of PPO activity was high in hexaconazole 1500 ml ha^{-1} compared to other treatments (Fig.12). No appreciable increase in PPO activity was observed between the standard fungicide treatments and control plants upto 72 h after pathogen inoculation.

Similar results were obtained in case of chilli plants (*i.e.*) hexaconazole 1500 ml ha^{-1} showed high induction of PPO activity followed by hexaconazole 1000 ml ha^{-1} compared to other treatments (Fig.13). Hexaconazole 500 ml ha^{-1} and other standard fungicide treatments were on par with each other in the induction of PPO activity. The data on table 32 clearly showed the significant induction of PPO activity between different treatments and time intervals.

4.8.3.1. Polyphenol oxidase (PPO) isoforms

Different PPO isoforms were observed in hexaconazole treatments, mancozeb and control plants against the powdery mildew pathogen. The induction of PPO isoforms in

different hexaconazole concentrations *viz.*, 1500 ml ha^{-1}, 1000 ml ha^{-1}, 750 ml ha^{-1} and 500 ml ha^{-1} was very high whereas mild and weak induction of PPO isoforms was observed in mancozeb and untreated control chilli plants (Plate 18).

4.9. Effect of hexaconazole 5 SC on phylloplane and rhizosphere microorganisms

4.9.1. Okra

Hexaconazole at four different concentrations *viz.*, 500, 750, 1000 and 1500 ml ha^{-1} was sprayed along with mancozeb (1 Kg ha^{-1}) as a check to determine its efficacy on phylloplane and rhizosphere microorganisms at different day intervals *viz.*, 0, 1, 3 and 5 days after spray. Phylloplane and rhizosphere microorganisms were isolated from okra plants by following serial dilution technique and the number of fungal and bacterial colonies were determined.

Hexaconazole 5 SC inhibited the phylloplane fungal population at different day intervals *viz.*, 0, 1, 3 and 5 days after spray. The fungal population was 4.0 x 10^{3} cfu on 5th day after spray at high concentration of 1500 ml ha^{-1}. However, this treatment was on par with control (untreated) recorded 3.0 x 10^{3} cfu on 5th day after spray. The total phylloplane fungal population ranged from 1.0 to 15.0 x 10^{3} at all the day intervals. Initially the fungal population was more (zero and first day) and further it decreased on 3rd and 5th day after spray.

The bacterial population increased with increase in the concentrations of hexaconazole with increase in day intervals (0, 1, 3 and 5 days after spray). The population was initially less ranging from 43.0 x 10^{5} to 60.0 x 10^{5} cfu (from the lowest to highest concentration of hexaconazole) and it ranged from 59.0 x 10^{5} to 81.0 x 10^{5} cfu respectively on 0th and

1st day after spray. However, it further increased on 3rd and 5th day after spray ranging from 72.0 x 10^5 to 120.0 x 10^5 cfu and 110.0 x 10^5 to 162.0 x 10^5 cfu respectively. The bacterial population was minimum in all the treated fungicides when compared to control ranging from 86.0 x 10^5 to 185.0 x 10^5 cfu respectively from zero day to 5th day after treatment (Table 33).

In case of rhizosphere, the fungal population varied between all the treatments at different day intervals (0, 1, 3 and 5 days). The fungal population reduced with increase in concentrations of hexaconazole 5 SC. But the population increased with increase in day intervals and ranged from 58.0 x 10^3 to 42.0 x 10^3 cfu (from the lowest concentration to highest concentration of hexaconazole) and 67.0 x 10^3 to 37.0 x 10^3 cfu respectively on zero and first day after spray. The population decreased from 63.0 x 10^3 to 34.0 x 10^3 cfu and 75.0 x 10^3 to 48.0 x 10^3 cfu respectively on third and fifth day after spray as against control ranging from 50.0 x 10^3 to 57.0 x 10^3 cfu respectively from zero day to 5th day after spray.

The rhizosphere bacterial population increased with increase in day intervals. The population increased in all the treatments as compared to untreated control. The maximum bacterial population was observed in hexaconazole at 1500 ml ha^{-1} ranging from 218.0 x 10^5 to 231.0 x 10^5 cfu as against control with 175.0 x 10^5 to 185.0 x 10^5 cfu respectively on 0th and 1st day after treatment (Table 34).

4.9.2. Chilli

The fungal population increased with increase in concentrations of hexaconazole. However it decreased with increase in day intervals. The fungal population ranged from 8.0 x 10^3 to 17.0 x 10^3 (from lowest to highest concentration of hexaconazole) and

7.0 x 10^3 to 17.0 x 10^3 cfu respectively on zero day and 1st day after spray. All the treated fungicides recorded less fungal population when compared to control. Hexaconazole at 1500 ml ha^{-1} treatment (18.0 to 24.0 x 10^3 cfu) was on par with control ranging from 15.0 x 10^3 to 21.0 x 10^3 cfu respectively on 0, 1 and 3 days after spray.

In case of phylloplane bacterial population, similar trend was noticed as in okra plants. The population increased with increase in concentrations of hexaconazole along with increase in day intervals. The maximum population was observed in hexaconazole treated at 1500 ml ha^{-1} recorded 47.0 x 10^5, 55.0 x 10^5, 110.0 x 10^5 and 121.0 x 10^5 cfu respectively on zero, 1, 3 and 5 days after treatment as against control which recorded 59.0 x 10^5, 71.0 x 10^5 and 95.0 x 10^5 and 116.0 x 10^5 cfu on zero, 1, 3 and 5 days after spray respectively (Table 35).

The rhizosphere fungal population was increased with increase in day intervals and it was found to be maximum in hexaconazole at 1500 ml and 1000 ml ha^{-1} recording 53.0 x 10^3, 79.0 x 10^3, 87.0 x 10^3 and 81.0 x 10^3 cfu and 47.0 x 10^3, 64.0 x 10^3, 73.0 x 10^3 and 96.0 x 10^3 cfu respectively on 0, 1, 3 and 5 days interval. However these two treatments were on par with control recording 48.0 x 10^3, 67.0 x 10^3, 97.0 x 10^3 and 112.0 x 10^3 cfu.

In case of rhizosphere bacterial population, there was no remarkable change in bacterial population between all the treatments at all the day intervals. Maximum population observed in hexaconazole treated at 1500 ml ha^{-1} among the different concentrations of hexaconazole treatment. However in the rhizosphere region of chilli, maximum population was observed in control (untreated) plants which recorded 227.0 x 10^5, 260.0 x 10^5, 277.0 x 10^5 and 296.0 x 10^5 cfu followed by mancozeb (1 Kg ha^{-1}) recording 215.0 x 10^5, 238.0 x 10^5, 255.0 x 10^5 and 248.0 x 10^5 cfu (Table 36).

4.10. Compatibility of hexaconazole 5 SC with biocontrol agents

The efficacy of hexaconazole at six different concentrations *viz.,* 0.001, 0.01, 0.05, 0.10, 0.5 and 1.0 ppm were tested against fungal biocontrol agents *viz., Trichoderma viride* and *T. harzianum* and bacterial biocontrol agents *viz., Pseudomonas fluorescens* (Pf 1) and *Bacillus subtilis*.

Among the different concentrations, the highest concentration of hexaconazole at 1.0 ppm effectively inhibited the mycelial growth of *T. viride* (3.10 cm), *T. harzianum* (3.60 cm) followed by 0.5 ppm (6.66 cm, 7.50 cm respectively) as against the control plates with the mycelial growth of 8.96 cm and 8.93 cm respectively (Table 37). A decrease in the mycelial growth was observed as the concentration increased, however the hexaconazole at lower concentrations *viz.,* 0.001, 0.01, 0.05 and 0.10 ppm were not inhibitory to these biocontrol agents and showed the similar growth as that of control plates (Plate 19a and b). The growth of bacterial biocontrol agents *viz., P. fluorescens* (Pf 1) and *B. subtilis* was not affected due to the spray of hexaconazole 5 SC (data not given).

4.11. Effect of hexaconazole 5 SC with other plant pathogens

The effect of different concentrations of hexaconazole 5 SC *viz.,* 0.001, 0.01, 0.05, 0.10, 0.50 and 1.00 ppm was tested against the pathogens *viz., Alternaria capsici* and *Colletotrichum capsici* under *in vitro* conditions. The highest concentration of hexaconazole was highly inhibitory to both the pathogens. *A. capsici* was significantly inhibited with the mycelial growth of 2.62 cm at 1.0 ppm concentration followed by 0.5 ppm (4.96 cm) as compared to control with the growth of 8.83 cm. The lower concentrations (0.5 and 0.1 ppm) also recorded the strong inhibition of mycelial growth

of *A. capsici*. However, the lowest concentration of hexaconazole (0.001 ppm) showed the similar mycelial growth as that of control plates (Table 38, Plate 20)

Similarly, *C. capsici* was also significantly inhibited with the mycelial growth of 2.43 cm at high concentration of 1.0 ppm followed by 0.5 ppm (4.36 cm) and 0.10 ppm (5.53 cm) as compared to control plate with the growth of 8.40 cm. Hexaconazole at lower concentrations (0.001, 0.01 and 0.05 ppm) showed the significant inhibition of mycelial growth of *C. capsici* as compared with the control plates (Table 38).

4.12. Residues of hexaconazole 5 SC on harvested okra, chilli fruits and soil

The residues of hexaconazole 5 SC were found at below detectable level in the harvested fruits of both okra and chilli (Table 39 and 40). The residues in soil was also found at below detectable level in both the crops. The standard chromatogram of hexaconazole and the linear curve are depicted in Fig.1. The mean recovery was 83.40 per cent at 0.5, 3.0 and 5.0 µg level of hexaconazole in okra crop and it was 83.51 per cent at 0.5, 3.0 and 5.0 µg level in chilli crop. The mean recovery of 82.86 per cent was observed in soil. The minimum detectable level was 0.020 µg g^{-1} for both okra and chilli fruits whereas it was 0.01 µg g^{-1} in soil (Table 41).

CHAPTER V

DISCUSSION

Hexaconazole 5SC, a novel protectant, curative, systemic triazole fungicide was used throughout the tenure of the present investigations. The study focussed on its bioefficacy against okra and chilli powdery mildew pathogens, phytotoxicity, spore germination, persistence, compatible with insecticides and biocontrol agents, efficacy on phylloplane and rhizosphere microflora, induced systemic resistance, biochemical changes and its residues in the harvested fruits. The results obtained from *in vitro* and *in vivo* condition are discussed hereunder.

5.1. Bioefficacy of hexaconazole 5SC

Powdery mildew disease of okra/bhendi caused by *Erysiphe cichoracearum* DC is prevalent in both field and greenhouse crops. It usually appears late in the season reaching its maximum intensity during fruit formation. The yield loss caused by this disease is varying from 17.0 to 86.6 per cent. No source of complete resistance to powdery mildew is available in the cultivated bhendi (Jhooty *et al*., 1977).

The currently available primary mildewcides are sterol demethylation inhibitors (DMIs) and the DMIs have been perceived by many growers as being highly effective for mildew control and several authors have indicated positive effects on one or more elements of yield by one or more of the DMI fungicides in the presence of mildew (Spotts and Cervantes, 1986; Yoder *et al*., 1985; Yoder *et al*., 1984 and Yoder *et al*., 1998). Powdery mildew disease can be kept under check with elaborate chemical spray programme (Sen and Kapoor, 1974; Kolbe, 1981). Chemical control by fungicide

spraying is the only method adopted to manage the powdery mildew disease. The fungicides that are being used are wettable sulphur (Sridhar and Sohi, 1973), thiophanate methyl (Datar, 1981) and carbendazim (Prakash and Raoof, 1982).

Systemic triazole fungicides namely hexaconazole (0.1%) or triadimefon (0.05%) introduced recently are found better for disease control (Tripathi and Singh, 1996). In the present investigations, a triazole compound of hexaconazole 5SC was evaluated for its bioefficacy against okra and chilli powdery mildew disease. Hexaconazole was tested at four different concentrations *viz*., 500, 750, 1000 and 1500 ml ha^{-1} in three field trials and greenhouse trials. Hexaconazole at the highest concentration of 1500 ml ha^{-1} was found to be effective against the okra and chilli powdery mildew disease both in field (Fig. 2 and 3) and greenhouse trials followed by hexaconazole at 1000 ml ha^{-1}, 750 ml and 500 ml ha^{-1}. The efficacy of hexaconazole at 500 ml ha^{-1} was comparable to mancozeb at 1 kg ha^{-1}. Increase in yield was also obtained from all the hexaconazole treatments when compared to other treatments in all the three trials (Table 3, 4 and 5). These results were in confirmity with the findings of several workers. Spray application with tridemorph (0.05%) or carbendazim (0.1%) or dinocap (0.1%) may be recommended for the control of powdery mildew disease of bhendi (Regupathy and Thamburaj, 1990). Spraying of carbendazim (0.2%) once or tridemorph (0.08%) followed by two dusting with sulphur at 10 days interval was found effective in controlling the powdery mildew (Gawande and Peshney, 1987). Hexaconazole was found to be effective over thiophanate methyl, calixin, chlorothalonil and wettable sulphur in controlling the grapes powdery mildew disease and in increasing the grape yield for two seasons (Varalakshmi *et al*., 1999). Dhurj *et al*. (1994) noticed considerable reduction in bud infection of rose powdery mildew with hexaconazole. Rao (1991) found earlier that 0.05 per cent Rubigan (Fenarimol) was effective against grapevine powdery mildew.

Hexaconazole was found to be highly effective and reduced the disease severity of cucumber powdery mildew (*Sphaerotheca fuliginea*) while dinocap, wettable sulphur and myclobutanil were least effective (Gupta and Amita Gupta, 2001). Wettable sulphur

was found to be effective but was phytotoxic to the cucumber foliage resulting in scorching of leaf margin. Due to such adverse effect, sulphur was not used against cucumber powdery mildew (Nene and Thapliyal, 1993).

A new formulation of hexaconazole (Contaf 5 SC) and its combination with captan was found to offer good control of powdery mildew of grapes (Shitole *et al*., 2000a), rust of sunflower (Sharma *et al*., 2000) and grape rust (Sharma *et al*., 1999). Shitole *et al*. (2000b) proved that the combination of hexaconazole and captan provided good control of powdery mildew of rose. Sivasankaran *et al*. (1991), in a three year study, recorded excellent control of the powdery mildew disease in mango with hexaconazole which was superior to triadimefon and wettable sulphur.

Chilli powdery mildew occurs severely under both field as well as green house conditions and causes severe defoliation. Mathur *et al*. (1972) reported earlier that chilli pepper (*Capsicum annuum*) was affected by *L. taurica* leading to severe defoliation and reduction in size and number of fruits and there was a 25 to 30 per cent increase in yield with the use of fungicides.

Apart from the chemical method no other method is sufficiently effective to ensure the protection of chilli against powdery mildew attack. In the present study, the bioefficacy of hexaconazole 5 SC was determined in chilli crop against powdery mildew disease from three field trials and greenhouse trials. Hexaconazole was tested at four

different concentrations *viz.*, 500, 750, 1000 and 1500 ml ha^{-1}. Chilli powdery mildew disease was effectively controlled at higher concentration of 1500 ml ha^{-1} followed by 1000 ml ha^{-1}, 750 ml ha^{-1} and 500 ml ha^{-1}. The efficacy of hexaconazole at 500 ml ha^{-1} was comparable with that of mancozeb at 1 kg ha^{-1}. Fruit yield was also increased at 1500 ml ha^{-1} of hexaconazole when compared to all other fungicide treatments. Chandrashekar and Sharma (1996) revealed that powdery mildew of pea was controlled more effectively by spraying hexaconazole with concomitant increase in green pod yield than by triadimefon, thiophanate methyl and wettable sulphur. Carbendazim (0.1%) spray was highly effective to reduce powdery mildew disease of mango followed by Anvil (0.1%) (Sharma, 1992).

Singh (2000a) who confirmed his findings that the chemical in its monosodium salt form was effective at 125 µg ml^{-1}. The powdery mildew disease development was checked in glasshouse at 2000 µg ml^{-1} by post inoculation treatment and the result was comparable with those of carbendazim (1000 µg ml^{-1}) and wettable sulphur (2000 µg ml^{-1}).

All the triazole fungicides *viz.*, propiconazole, penconazole, hexaconazole and triadimefon performed better as compared to conventional fungicides *viz.*, tridemorph, dinocap and wettable sulphur against the powdery mildew of fenugreek caused by *Leveillula taurica* (Dhurj *et al.,* 1999). For the management of powdery mildew of fenugreek, the spraying of wettable sulphur, dinocap and triadimefon have been reported by Rathore and Rathore (1995). Pankaj Audichya and Thakore (2000) revealed that carbendazim, triadimefon and iprobenfos applied as protective fungicides gave more than 50 per cent control of powdery mildew disease in opium poppy but maximum control was found in case of carbendazim.

Fungicides *viz*., cyperconazole, flusilazole, penconazole and hexaconazole provided post-infection activity of pea rust for more than 72 h followed by difenoconazole, triadimefon and fenarimol while mancozeb and chlorothalonil were least effective with 100 per cent disease incidence (Gupta and Shyam, 2000). Also the efficacy of fungicides like hexaconazole, triadimefon, difenoconazole and flusilazole in the management of pea rust disease has already been reported in protective spray programme (Gupta and Shyam, 1998).

New systemic fungicides *viz*., Contaf 5E, Contaf 5SC and hexaconazole + captan were effective in the control of fruit rot in chilli with simultaneous increase in the yield (Sharma *et al*., 1998). Raghavendra (1996) had reported that systemic fungicides *viz*., carbendazim, bitertanol and triadimefon were found to be effective at 1000 ppm concentration against the fruit rot in chilli.

Senthil *et al*. (2000) revealed that the fungicide hexaconazole was effective against soybean rust pathogen. The superiority of hexaconazole over triadimefon and Bordeaux mixture in coffee leaf rust control was reported by Daivasikamani and Govindarajan (1989) and Chandra Mouli *et al*. (1997).

The investigation of Sharma *et al*. (2000) clearly indicated that the spraying of hexaconazole + captan combination effectively managed the leaf rust disease in sunflower. For the control of tomato early blight disease, either Dodine (0.3%) or Difenoconazole (0.1%) have not only recorded significantly low disease, but also resulted in increased yield (Nagaraja *et al*., 1999).

Triazole compounds *viz*., hexaconazole, flusilazole, tebuconazole, difenoconazole, propiconazole and other compounds are the members of azole class of sterol biosynthesis inhibitors which exert their fungitoxic effects by inhibiting the 14 α-methyl sterol and 14 α-demethylase enzyme system involved in sterol biosynthesis (Vanden Bosch, 1988). Inhibition of 14 α-demethylase enzyme by the azoles leads to accumulation of 14 α-methyl sterols and the depletion of usual sterols required for fungi and this will lead to disrupt the membrane function and finally death of fungal cells occur. Since hexaconazole 5 SC was found to be highly effective against the powdery mildew disease of okra and chilli, inhibition of sterol biosynthesis in *E. cichoracearum* and *L. taurica* by this chemical prove the possible mechanism of action behind its effectiveness. The other compounds belong to triazole family were also reported to inhibit the 14-α-demethylase enzyme and affect the sterol composition of several fungi (Kwok and Loeffler, 1993).

5.2. Compatibility of hexaconazole 5 SC with insecticide

The combination treatment of both fungicide and insecticide was good in reducing the pests and diseases of many crops. Mixture of two pesticides may produce a greater insecticidal action than the sum of their individual components by exhibiting synergism (Gera, 1973), thus minimising the pesticidal load on the environment and also bring about significant cost efficiency (Hewlett, 1961).

The present study revealed that the combination treatment of hexaconazole at 500 ml ha^{-1} with monocrotophos at 1000 ml ha^{-1} was effective with the least population of whitefly and leaf hopper in okra and with the least thrips population and aphid damage in chilli as compared to individual insecticide application. Also the combination treatment of hexaconazole 500 ml ha^{-1} with mancozeb 1 kg ha^{-1} showed the least per cent disease index of powdery mildew both in okra and chilli crop (Table 9 and 10; Fig. 4 and 5). These results are

in confirmity with the findings of Varalakshmi *et al*. (2000) who concluded that hexaconazole along with monocrotophos (500 ml + 1000 ml ha^{-1}) was significantly superior over all other treatments in controlling the pest damage (thrips, flea beetle) and powdery mildew disease with increase in grapevine yield.

Johnson (2001) reported that hexaconazole at 2000 ml ha^{-1} was highly compatible with monocrotophos and showed synergistic action. Rice greenleaf hopper population, rice leaf folder damage and sheath blight incidence were reduced due to combined treatment of hexaconazole and monocrotophos. Also due to this combined effect, the leaf hopper population, leaf miner damage and late leaf spot disease incidence in groundnut were reduced. Combined application of Rubigan with mancozeb/phosphamidon did not affect the fungicidal efficacy of Rubigan and there was no phytotoxicity symptoms also. However, there was no incidence of powdery mildew or other sucking pests of grapes in all the treatments (Balamurali Krishnan and Jeyarajan, 1998).

Sharma *et al*. (1999) found that the combination of hexaconazole + captan (0.2% + 0.2%) has performed better than systhane, when the grape rust disease pressure was high. The foliar spray of monocrotophos has been proved effective and recommended to control the insect pests *viz*., jassids, fruit and shoot borer (Singh, 2000b). The new formulation of hexaconazole (Contaf 5 SC) and the combination of hexaconazole with captan were found to be effective against powdery mildew in grapes (Shitole *et al*., 2000a) and rust in sunflower (Sharma *et al*., 2000). The mixtures of hexaconazole with captan (1: 17.5) and with mancozeb (1:28) were also highly effective for controlling white rust on chrysanthemum. No undesirable phytotoxic effects were evident on chrysanthemum and rose plants treated with hexaconazole or its combination (Lam and Lim, 1993). Sharma *et al*. (1998) indicated

that the new systemic fungicides *viz*., Contaf 5E, Contaf 5 SC and hexaconazole + captan were effective for the control of fruit rot in chilli besides increasing the yield.

5.3. Persistence of hexaconazole 5 SC in okra and chilli fruits

Control of powdery mildew caused by *E. cichoracearum* and *L. taurica* in okra and chilli respectively constitutes a major operation in cultivation of both the crops. Several fungicides belonging to triazole group have been introduced in India to control diseases of fruits and vegetable crops particularly apple scab and powdery mildew (Gajbhiye *et al*., 1995). Hexaconazole, a preventive as well as curative triazole fungicide is recommended for the control of powdery mildew in okra and chilli crops due to its effectiveness against this disease. Since powdery mildew occurrence was late in the season particularly during the fruit formation, this type of systemic chemical should be sprayed when the disease pressure is high and there will be chance for persistence of this chemical on plant/plant parts or fruits.

Persistence of protective fungicide on the surface of the plant/plant parts plays an important role in determining their disease reduction potential and was highly useful in developing spray schedules. In the present study, the persistence of hexaconazole 5 SC at four different concentrations *viz*., 500 ml, 750 ml, 1000 ml and 1500 ml ha^{-1} was determined both in okra and chilli crop. The results indicate that the persistence of hexaconazole 5 SC was found up to seven days in all the trials of both the crops and maximum amount of residues persisted at higher concentration of 1500 ml ha^{-1}. The amount of residues was initially (0th day) high and thereafter it was dissipated. The amount of residues detected was maximum for higher concentration of hexaconazole (Table 11 and 15; Fig.6 and 7). The recovery of hexaconazole was high in okra crop when compared to chilli crop which may be due to genetic make up of the crop or due to temperature difference. Since hexaconazole 5 SC is highly effective against the powdery mildew disease of both okra and chilli, it is clear

from this investigation that the persistence of this chemical in fruits is only up to seven days and therefore the fruits are safe for consumption and free from residues at harvest.

These results are in agreement with the findings of Varalakshmi *et al*. (1999) and (2000) who found that hexaconazole neither persists in the soil nor in fruits after 10 days of spraying till harvest. They also included that there was a gradual decrease in the residue content with the increase in the time interval. The residue level in fruits was at below detectable level from seventh day until the time of harvest.

Johnson (2001) also reported that hexaconazole residues were recovered from rice and groundnut up to seven days and after that, the residues were at below detectable level in both rice and groundnut. He also indicated that the amount of residues recovered was increased with increase in concentration of the chemical. Senthil *et al*. (2000) reported the hexaconazole did not persist in soil, seed kernel, oil and deoiled cake.

Residues of flusilazole and hexaconazole showed higher degree of antisporulant activity against cucumber powdery mildew for a long period which checked the build up of secondary inoculum Gupta and Amita Gupta (2001). They also concluded that the long persistent effect of flusilazole and hexaconazole could be used in planning the spray programme against cucumber powdery mildew. Flusilazole, a triazole systemic fungicide was effective against apple powdery mildew, scab and grapes powdery mildew. The flusilazole residues persisted up to 15 days at 40 and 80 g a.i ha^{-1} rate of application (Gajbhiye *et al*., 1995) and the half-life was found to vary between 2.2 to 2.8 days.

Fenarimol residues persisted in grapes to the maximum of 14 days after last spray and dissipation rate was more during summer since the chemical was highly effective against grapevine powdery mildew (Balamurali Krishnan and Jeyarajan, 1998). Naseema Beevi *et al.* (1996) reported that the mean residues reduced to below detectable level by 7th day of application. They also reported that the progressive decay of mancozeb residues in bittergourd fruits over a period of time took place at the half life of 2.27 and 2.53 days.

Carbendazim persistence in apples was observed up to 30 days when it was dipped in 500 ppm solution in cold storage when compared at ambient temperature storage (Bharat and Sharma, 1997). The initial deposits of carbendazim were lower in apple fruits than in leaves. Dissipation during the subsequent 5 day intervals declined steadily with progress in time. On fruits, the residues could be detected up to 10 days, whereas on leaves, the residues even persisted beyond this period as suggested by Sharma *et al.* (1997).

Three formulations of mancozeb *viz*., Dithane M-45 persisted up to 24 days in leaves and 20 days in fruits; Dhanuka M-45 and Luze M-45 persisted up to 20 and 16 days in apple leaves and fruits respectively (Gupta *et al*., 1994). Similarly Jhooty and Munshi (1976) have also reported the persistence of mancozeb in tomato leaves up to 7 days.

Persistence of chlorothalonil on tomato crop was up to 15 days and on mustard, it was up to 45 days and the half-life of chlorothalonil was 2.8 to 3.6 days on tomato and 6.0 to 8.8 days on mustard. This was suggested and reported by Gajbhiye *et al.* (1996). Dithane M-45 persisted for longer period in glass house than in the field which was probably due to different environmental conditions (Thind and Jhooty, 1982).

Systemic fungicides like Aliette applied against *Alternaria alternata* persisted in roots and leaves of tomato seedlings up to 23 days while thiophanate methyl and carbendazim remain persisted for 19 days after soil application (Chandravanshi *et al*., 1994). Vyas and Jain (1977) reported little longer persistence (27 days) of carbendazim in tomato and brinjal seedlings. Vijayalakshmi *et al*. (2000) reported that the residues of triazophos reached below detectable level within 10 days after spray and a waiting period of 5.0 and 9.0 days are required before harvesting the okra fruits.

5.4. Dissipation of hexaconazole residues in okra and chilli

Dissipation of hexaconazole residues during the subsequent five day intervals declined steadily with progress in time both in okra and chilli fruits. A slower rate of dissipation of hexaconazole residues in okra during the second and third trial may be due to temperature difference (Table 12 and 16).

Seven different transformations were used for representation of degradable hexaconazole residues Vs time and among them, the regression (r) was significant for hexaconazole in okra and chilli (Table 13 and 17). The residues decline at a faster rate as it follows the first order kinetics with the half life ($T_{0.5}$) of 2.12 to 2.98 days in okra (Table 14) and 1.90 to 3.10 days in chilli (Table 18). The safe waiting period of 15 days and 11 days are required before harvesting the okra and chilli fruits, can be considered safe for consumption (Table 14 and 18). It can be concluded that the hexaconazole at the recommended dose involves low health risk and a safe waiting period of 15 and 11 days could be recommended for use of the hexaconazole on okra and chilli crop.

5.5. Phytotoxicity of hexaconazole 5 SC

The present investigations revealed that there was no phytotoxicity or any injury on okra and chilli crop even with a higher concentration of hexaconazole 5 SC (1500 ml ha^{-1}) (Table 19 and 20). This was supported by the findings of Varalakshmi *et al*. (2000), in which there was no report of leaf injury on grapevine at a higher concentration of 0.2 per cent hexaconazole. Johnson (2001) reported that there was no phytotoxicity or any leaf injury on rice and groundnut crops due to hexaconazole treatment. There was no undesirable phytotoxic effects evident on chrysanthemum and rose plants treated with hexaconazole or its mixtures (hexaconazole + captan (or) with mancozeb) (Lam and Lim, 1993).

5.6. Spore germination

The present study indicates that hexaconazole at higher concentration of 2000 ppm showed the least per cent spore germination of *E. cichoracearum* and *L. taurica* (Fig. 8 and 9) From this study, immediately after hexaconazole treatment, the per cent spore germination was maximum and it was further reduced on subsequent day intervals (Table 23 and 24). This is in agreement with the findings of Varalakshmi *et al*. (1999) who revealed that even at a concentration of 100 ppm, it was able to reduce the spore germination of *Uncinula necator*.

5.7. Biochemical changes

In the present study, the chlorophyll content (*a, b* and total chlorophyll) increased in hexaconazole treated okra and chilli plants when compared to untreated control plants. Maximum chlorophyll content was recorded at 72 h after spray when the plants were treated with highest concentration of hexaconazole (1500 ml ha^{-1}) (Table 25 and 26). These findings are in confirmity with the results of Johnson (2001) who reported that the hexaconazole spray increased the chlorophyll (*a, b* and total) content in rice and groundnut leaves.

It was reported earlier that various biochemical changes occurred during infection by various plant pathogens (Gerwitz and Durbin, 1960; Krog *et al*., 1961; Vidhyasekaran and Krishnaswamy, 1974). The content of *a, b* and total chlorophyll decreased with the rust disease development in the leaves of susceptible and tolerant varieties of safflower when compared to healthy leaves. In general, the chlorophyll content was higher in tolerant varieties as compared to susceptible one. (Reeti Singh *et al*., 1998). Reduction in chlorophyll might be due to distribution of chlorophyll or higher accumulation of sugar (Curtis and Clark, 1950) which would make the nitrogen unavailable for chlorophyll formation by binding it for the formation of protein (Subramanyam *et al*., 1976).

Total chlorophyll, chlorophyll *a*, chlorophyll *b* and carotenoids in leaves of cowpea plants raised from carbendazim treated seeds in soil inoculated with *Rhizoctonia solani* and *R. bataticola* were significantly higher than the untreated ones. The amount of chlorophyll *a* was significantly higher than that of chlorophyll *b* (Shahina Kalim *et al*., 2000).

Treatment of plants with a variety of chemicals *viz*., fungicides, herbicides and growth regulators sometimes leads to accumulation of phenolic compounds (Bajaj, 1988). The antifungal activity of some fungicides is also attributed to their metal chelating ability (Kataria *et al*., 1981). In the present study, the total phenol and soluble protein content was determined in okra and chilli plants and observed that all the treated fungicides showed significant increase in phenol and protein content as against control unsprayed plants. The total phenol and soluble protein content was significantly increased in hexaconazole treated okra and chilli plants at higher concentration especially at 72 h after treatment (Table 27 and 28; Fig.10 and 11).

These findings are in agreement with Johnson (2001), who found that the hexaconazole spray (2000 ml ha^{-1}) increased the soluble protein and total phenol content in rice and groundnut leaves over the control. Increase in phenolic compounds have also been observed earlier in mustard and chickpea plants with the application of carbendazim and carboxin (Kotastane and Vyas, 1992; Singh and Sindhan, 1998).

The healthy plants of tolerant varieties of safflower had higher amount of total and ortho-dihydric phenols than the susceptible cultivar (Reeti Singh *et al*., 1998). Disease development lowered the phenolic concentration in the upper leaves than of tolerant and susceptible cultivar. Patil and Kulkarni (1977) and Mathar and Vidhyasekaran (1978) also observed reduced phenol content of sunflower after infection by *Puccinia helianthi*. Total phenol, reducing sugar, macroelements and microelements concentration except Fe were induced with carbendazim seed treatment against *Rhizoctonia* of cowpea in comparison to control (Shahina Kalim *et al*., 2000).

In addition to common fungicides, some laboratory chemicals have been reported to control the plant pathogens effectively (Hait and Sinha, 1986). Soybean leaves sprayed with alum, dipotassium hydrogen phosphate against anthracnose caused by *Colletotrichum truncatum* showed increase in protein content compared to control (Chandrasekaran and Rajappan, 2001). Increased protein synthesis in the host was correlated with disease resistance in oats against *Puccinia coronata* (Yamamoto and Tani, 1982), in wheat against *Helminthosporium sativum* (Hait and Sinha, 1986) and in rice against *Sarocladium oryzae* (Sankar and Sinha, 1989). Increase in soluble protein can directly be correlated with defense inducing phytoalexin (Partridge and Keen, 1976).

5.8. Induction of defense related enzymes

A new strategy of chemically induced disease management in which activation of the plant's own defense system has been reported. Chemicals *viz*., salicylic acid, 2,6-dichloroisonicotinic acid, benzothiadiazole (BTH) and 3-amino butyric acid are known as potent inducers of plant defense system (Cohen, 1994; Vernooij *et al*., 1995; Gorlach *et al*., 1996; Spletzer and Enyedi, 1999).

Foliar spray of fungicides or phosphate and potassium salts can induce systemic protection against foliar pathogens particularly powdery mildew in various crops such as cucumber, maize, rose, grapevine, apple, mango and nectarines. In the present investigation, induction of phenylalanine ammonialyase, peroxidase and polyphenol oxidase activity due to the spray of hexaconazole in okra and chilli plants was observed. This study revealed that there was significant increase in PAL enzyme activity between the different day intervals both in okra and chilli crop however, there was no remarkable change reported in PAL enzyme activity both in inoculated and uninoculated chilli leaves. Maximum PAL enzyme activity was observed in the highest concentration of hexaconazole in inoculated and uninoculated plants (Table 29 and 30).

These results are in confirmity with the findings of Subhas Chander (1992), who reported that the activity of phenylalanine ammonialyase (PAL) was higher in the resistant variety of chilli than in the susceptible variety (susceptible to *Leveillula taurica*). Arvinder Kaur and Kolte (2001) reported that benzothiadiazole (BTH) treatment had no significant effect on phenylalanine ammonialyase activity in mustard plants against white rust caused by *Albugo candida*. BTH as a foliar spray provided protection in wheat against powdery mildew caused by *Blumeria graminis f.sp. tritici* for the entire season and this chemical has been shown to activate resistance in several plants including

Arabidopsis, cucumber and tobacco (Benhamou and Belanger, 1998; Friedrich *et al*., 1996; Lawton *et al*., 1996).

Salicylic acid has been reported as a possible signal molecule and its exogenous application induces resistance and PR-proteins which typically accompany Systemic Acquired Resistance (White, 1979; Ward *et al*., 1991). A single spray of phosphate induced a high level of systemic protection against powdery mildew caused by *Sphaerotheca fuliginea* in cucumber (Reuveni *et al*., 1993 and 1995). Phosphate salts induced immunization of cucumber plants against various diseases including powdery mildew (Gottstein and Kuc, 1989; Mucharromah and Kuc, 1991).

In the present study, peroxidase and polyphenol oxidase activity were observed to be increased in okra and chilli plants when these plants were sprayed with hexaconazole against powdery mildew disease. The peroxidase and polyphenol oxidase activity were significantly increased at all the time intervals in all the concentrations of hexaconazole treated okra and chilli plants however those activities were maximum at 1500 ml ha^{-1} of hexaconazole. All the treated fungicides showed higher activity of peroxidase and polyphenol oxidase in okra and chilli plants (Table 31 and 32; Fig.12 and 13).

The native gel electrophoresis of chilli samples showed variations in the induction of peroxidase and polyphenol oxidase isoforms with respect to different concentrations of hexaconazole. These results are in agreement with the findings of Johnson (2001), who reported significant induction of peroxidase activity in groundnut plants when sprayed with hexaconazole 5SC.

The activity of peroxidase was found to be high in the resistant variety, IIHR 517A of chilli and in improved variety, Pusa Jwala as reported by Subhas Chander (1992). Higher peroxidase activity has been correlated with disease resistance in many plants (Vidhyasekaran, 1988).

Peroxidase and β-1,3 glucanase are related to cross-linking of cell wall components, polymerization of lignin and suberin monomers and subsequent resistance to pathogens in several host-pathogen interactions (Reuveni, 1995). Mustard leaves sprayed with BTH (benzothiadiazole) three days prior to inoculation with *A. candida* showed elevated levels and enhanced activity of peroxidase at 11 days after inoculation (Arvinder Kaur and Kolte, 2001). Earlier several workers have shown the correlation of phenol compounds and peroxidase enzyme involved in lignin biosynthesis, production of toxic quinones and phytoalexins with the onset of resistance (Glazener, 1982; Hammerschmidt *et al*., 1982; Daayf *et al*., 1997).

Shahina Kalim *et al*. (2000) reported that there was increase in the specific activity of polyphenol oxidase, peroxidase and reduction in specific activity of catalase in roots of plants raised from carbendazim treated seeds in comparison to untreated ones. Peroxidase specific activity was several times more than polyphenol oxidase specific activity. Therefore the increased activities of polyphenol oxidase and peroxidase along with higher amount of total phenol might have contributed enhanced host resistance.

5.9. Effect of hexaconazole 5 SC on phylloplane and rhizosphere microorganism

In the present study, changes in microbial population of the phylloplane and rhizosphere at different day intervals in okra plants due to the spray of hexaconazole

5 SC was studied and the results revealed that the bacterial population increased with increase in the concentration of hexaconazole, whereas the fungal population in the phylloplane was high on zero and 1st day and later it decreased on 3rd and 5th day after spray. The phylloplane bacterial population increased with increase in day intervals and found to be minimum as compared to control. In rhizosphere region also, reduction in the fungal population with increase in concentration of hexaconazole was noticed. The bacterial population in rhizosphere increased in all the treatments as compared to control okra plants (Table 33 and 34).

Similar type of results were observed in chilli crop in which the phylloplane fungal population decreased in all the treated fungicides as compared to control. In rhizosphere fungal and bacterial population, no significant difference was observed between all the treatments and day intervals (Table 35 and 36).

The present investigations are in agreement with the results of Johnson (2001), who found that the rice phylloplane fungal and bacterial population decreased initially and reached a maximum on subsequent day intervals. The rhizosphere fungal and bacterial population decreased initially and reached maximum on seventh day after spray. In groundnut also, the phylloplane fungal population was completely inhibited initially and further it increased and the bacterial population was also reduced initially and later increased.

5.10. Compatibility of hexaconazole with biocontrol agents

Biocontrol agents have been used with fungicides without any toxic effect on antagonists (Papavizas and Lumsden, 1980). Use of some strains of *Trichoderma harzianum* Rifai tolerant to fungicides have also been reported for the integrated control of plant diseases

(Papavizas and Lewis, 1981; Papavizas *et al*., 1982). Therefore different fungicides were evaluated at various concentrations to know the tolerance limit of the bioagent.

The present investigation were made in connection with the effect of different concentrations of hexaconazole 5SC on fungal bioagents *viz*., *Trichoderma viride* and *T. harzianum* and bacterial antagonists *viz*., *Pseudomonas fluorescens* (Pf 1) and *Bacillus subtilis*. From this study it is clear that hexaconazole 5 SC at 1.0 ppm was highly inhibitory to the growth of both *T. viride* and *T. harzianum* observed at two different day intervals (3rd and 5th day).

However the lower concentratons of hexaconazole were not inhibitory to *T. viride* and *T. harzianum* (Table 37) and showed similar mycelial growth as that of control. The bacterial antagonists *viz*., *Pseudomonas fluorescens* (Pf 1) and *Bacillus subtilis* did not show any effect on different concentrations of hexaconazole. Most of the fungi require sterols for their growth. Since hexaconazole as a sterol biosynthesis inhibitor inhibits the C_{14} demethylation in sterol biosynthesis of fungi leading to membrane disfunction and cell death (Buchenauer, 1987). Moreover the bacteria are not deprived of sterols and therefore hexaconazole showed no detrimental effect to the bacterial biocontrol agents. These findings are in agreement with the results of many workers. Johnson (2001) found that the hexaconazole effectively inhibited the mycelial growth of fungal antagonists *viz*., *T. viride, T. harzianum* and *Gliocladium virens* at a concentration of 0.5 ppm whereas the mycelial growth was completely inhibited at 5 ppm level.

Metalaxyl at 0.1 per cent and carbendazim (0.0065%) appear to be safe tolerance limit (ED_{50}) for the bioagent, *T. harzianum* however there was increasing trend of inhibition to the growth of *T. harzianum* at high concentration of the fungicides *viz*., metalaxyl and

carbendazim (2578 and 354 µg ml^{-1} respectively) as suggested by Sharma *et al*. (2001). Similar results were obtained for carbendazim and benomyl (Papavizas *et al*., 1982 and Viji *et al*., 1997) and for metalaxyl (Mukhopadhyay *et al*., 1986). Similar trend of tolerance limit was found for chlorothalonil (Abd-el Moity *et al*., 1982), captan and captafol (Papavizas, 1980) and thiram, mancozeb, captafol (Desai and Schlosser, 1994).

Ortiz Molinuevo *et al*. (1996) found good growth of *T. harzianum* at low and medium concentration of captan and no growth with carbendazim and benomyl was observed. The four fungal antagonists *viz*., *Chaetomium globosum*, *Trichoderma harzianum*, *T. viride* and an unidentified *Trichoderma* isolate were sufficiently insensitive to captan, mancozeb and thiram under *in vitro* studies suggesting that they could be successfully integrated with these fungicides in a field situation without detrimental effect on the biocontrol agents (Kay and Stewart, 1994).

Kolase *et al*. (2001) revealed that the fungicides *viz*., carbendazim, thiram and copper oxychloride variably influenced the survival of antagonists. Thiram had maximum adverse effect on the antagonists and its presence in soil drastically reduced from initial inoculum. The reduced population of *Trichoderma* in thiram treatment was in accordance with the results of Papavizas and Lewis (1981). Munnecke *et al*. (1981) stated that *Trichoderma spp*. was the common antagonist to appear after soil fumigation.

The application of antagonists along with fungicides did not hinder the cellulolytic enzymes secreted by antagonistic organisms particularly, *Trichoderma spp*. which are the most important biocontrol tools and the easy entry of antagonist into pathogen through penetration with direct lysis and degradation of cell wall (Chet and Baker, 1981). Copper oxychloride and carbendazim enhanced the cellulolytic enzyme production by the antagonists at lower

concentration (Karpagavalli and Ramabadran, 2001). Grinstein *et al*. (1979) reported that the integration of lower dose of fungicides with *Trichoderma* improved the disease control. Integration of metalaxyl with *T. harzianum* for the control of *Pythium* damping off in sugarbeet was reported by Sawant and Mukhopadhyay (1990).

When the biocontrol preparation (*Trichoderma spp*.) was applied along with dicarboximide fungicides *viz*., iprodione or vinclozolin, a trend towards better control was resulted leading to 84 to 91 per cent disease reduction (*Botrytis cinerea*) as compared with the control (Elad *et al*., 1993). Gullino *et al*. (1991) who sprayed dicarboximide + thiram in alternation with *Trichoderma* in order to control grey mould in tomato and strawberries.

5.11. Effect of hexaconazole on other pathogens

In the present study, hexaconazole 5 SC showed maximum inhibition to *Alternaria capsici* and *Colletotrichum capsici* at higher concentrations (1.00 ppm and 0.5 ppm). However, this chemical showed good inhibition to *C. capsici* in all the concentrations when compared to *Alternaria capsici* (Table 38).

Johnson (2001) also reported that the hexaconazole was highly effective against *Rhizoctonia solani* and *Sclerotium rolfsii*, moderately effective against *Helminthosporium oryzae* at 0.5 ppm and all the three fungi were completely inhibited at 5 ppm concentration level of hexaconazole. *Sarocladium oryzae* was highly sensitive while *Pyricularia oryzae* was moderately sensitive at 0.5 ppm level of hexaconazole and both were completely inhibited at 10 ppm level of hexaconazole. The new systemic fungicides *viz*., Contaf 5E, Contaf 5 SC and hexaconazole + captan were effective in the control of fruit rot in chilli and increased the yield (Sharma *et al*., 1998). Sudhakar *et al*. (1993)

found that hexaconazole was the best among all the fungicides tested against sheath blight of rice followed by propiconazole and iprodione.

Hexaconazole was effective against soybean rust (Senthil *et al*., 2000), grape rust (Sharma *et al*., 1999), coffee leaf rust (Chandra Mouli *et al*., 1997) and sunflower rust (Sharma *et al*., 2000) pathogens. Mcquilken *et al*. (1988) reported that hexaconazole and triadimenol were highly effective against cocoa pathogen, *Crinipellis perniciosa* under *in vitro* conditions.

5.12. Residues of hexaconazole 5 SC on harvested fruits of okra, chilli and in soil

Fungicides belonging to sterol biosynthesis inhibitors, acylalanines and triazole groups are successful in controlling several plant diseases but their excessive, irrational and indiscriminate use can pose problems pertaining to the safety of the consumer. There may be serious residue problems especially when these are applied at the maturing stage and a minimum waiting period is not followed. As many of the fruits and vegetables are consumed as raw products, fungicide residues on them may lead to health related problems. Work done on residues of fungicides in India is meagre although a number of fungicides are being used at present. The residue levels in the soil or edible parts vary with the dose of the fungicides used and with the total number of sprays done (Tripathi *et al*., 1976; Mithyantha *et al*., 1977). If the dose used is high and it is applied at the improper time and a total number of sprays exceed than the recommended ones, there is every chance that the residues left in the crops at harvest time are higher than the tolerance limits prescribed.

Standardization of fungicidal residue is an important activity as the quality parameters are interlinked with inherent toxicity, residual effects and phytotoxicity, etc. Analytical methods are given higher attention in order to ensure a higher degree of

repeatability and reproducibility. The most widely used methods for faster, easier and sensitive analysis that permit screening of a large number of samples include GC and HPLC.

The present study illustrated with the reports of hexaconazole residues on okra and chilli fruits and in soil. The hexaconazole residues were found at below detectable level in the harvested fruits of okra and chilli and in case of soil, it was found at below detectable level (Table 39 and 40) in both the crops. The standard chromatogram and linear curve of hexaconazole 5SC are given in Fig 1. From the fortified okra fruit samples, the mean recovery was 83.40 per cent and in chilli it was 83.51 per cent at 0.5, 3.0 and 5.0 µg level. In soil, the mean recovery was 82.86 per cent (Table 41). The minimum detectable level in okra and chilli fruit was 0.020 µg g^{-1} and in soil, it was 0.01 µg g^{-1} as the sample weight of fruits and soil were 25 g and 50 g respectively.

Johnson (2001) reported that the residues of hexaconazole were at below detectable level in harvested produces of rice (straw, grains and husk) and groundnut (kernels and shells). The FAO and WHO organizations jointly proposed the maximum residue limits (MRL) of hexaconazole between 0.05 to 0.1 mg kg^{-1} for various crops (Anon., 1990).

Varalakshmi *et al*. (1999) found that the average recovery of hexaconazole from the grape sample fortified at 1.0 ppm level was 83.3 per cent and they concluded that the residues of hexaconazole were found at below detectable level in soil and in fruits after 10 days of spraying. Varalakshmi *et al*. (2000) also reported that the recovery of hexaconazole in grapes fortified at 1, 2 and 5 ppm was more than 80 per cent and they found that the residue levels in fruits and soil were below detectable level (*i.e*) 0.04 µg g^{-1}

and 6.02 μg g^{-1} respectively. Senthil *et al*. (2000) revealed that the seed kernel, oil and deoiled cake of soybean were free of hexaconazole residue at the time of harvest.

Iprobenphos residues were not observed during the harvest of dry chilli fruits and residues remained in green chilli fruits up to 12 to 15 days (Sharma and Thakore, 1999). They also reported that the residue of chemical iprobenphos could not be detected in any treatment after 28 days of last spray.

Carbendazim residues persisted throughout the ripening period of the fruits and dissipated with a half life of 19 days in the whole mango fruit at room temperature and about 40 days at lower temperature. Captan residues on the other hand persisted for less than 15 days with a half-life of only 2.3 days at room temperature and for less than 30 days at lower temperature with a half life of 2.5 days (Awasthi and Debi Sharma, 1997).

Carbendazim residues on apple fruits could be detected up to 10 days, whereas on leaves, these even persisted beyond this period (Sharma *et al*., 1997). They concluded that carbendazim at the recommended dose involves low health risk at post-harvest stage, with a safe waiting period of 3 days. Bharat and Sharma (1997) reported that the persistence of carbendazim up to 30 days on apples at ambient temperature and further observed that residual half life and persistence of carbendazim was more in cold storage than at ambient temperature. Hargreaves (1983) has reported 20 times more residue of benomyl in the peel than in the pulp of litchi fruits.

Grapes are free from fenarimol residues at the time of harvest and these findings revealed that dissipation rate was more during summer when fenarimol residues reached

below detectable level even within a week after the last spray (Balamurali Krishnan and Jeyarajan, 1998). Hedider *et al*. (1987) also reported that during harvest time, the residues of fenarimol did not exceed the maximum permitted level of 0.1 μg ml^{-1} in chillies when the last application was seven days before harvest. Cabras *et al*. (1987) reported that residue level in grapes was related to weather conditions and the lowest values were found in hot dry areas.

Gajbhiye *et al*. (1995) found that no residues of flusilazole were detected in apple juice and a safe waiting period of 4 days was required for residues to fall below its MRL (Maximum Residue Limit-0.2 ppm). No residues of chlorothalonil were detected in seeds of both tomato and mustard at harvest (Gajbhiye *et al*., 1996). They suggested that the application of chlorothalonil at recommended rates is considered safe if the crop is to be raised for seed production. However, if the crop is raised for vegetable purpose, a safe waiting period of 15 days may be prescribed.

The progressive decay of mancozeb residues in bittergourd fruits over a period of time took place at the half life of 2.27 and 2.53 days and the waiting period of 2 and 4 days was recommended based on the maximum residue limit of 3 ppm as suggested by Naseema Beevi *et al*. (1996).

Ethylene thiourea (ETU) residues in grapes was analysed and reported in and around Hyderabad by Aillakananda Kakati and Jhelum (1993) revealed that the grapes available in and around Hyderabad could be considered as generally free from ETU residues.

Sharma *et al*. (1996) indicated that the fast dissipation of dodine on fruits of apple may be attributed to the dilution factor as the fruits were not fully developed. The other factor for less persistence of dodine may be the washing effect due to heavy rainfall. The residues of dodine could be detected up to 20 days in fruits and 30 days in leaves of apple but at the time of harvest, the residues disappeared rapidly. The safe waiting period was 14 days for the harvest of dodine treated apples.

Detectable level of mancozeb residues were noticed in fruits of tomato from the crop protected with 3 sprays of mancozeb and these residues dissipated to nearly 50 per cent level within one day and to the extent of 99 per cent at 15 days after last spraying (Jayakumar *et al*., 1995). They also concluded that the fruits harvested 2 days after third spray can be considered fit for consumption and a waiting period of 2 days can be advocated in this crop. Bhupal Reddy *et al*. (1993) indicated that the grapes can be consumed only after a lapse of 20 days following the last fungicide spray (Ethylene bisdithiocarbamate) preferably at a lower dose and washing with water prior to consumption.

Degradation of fungicides on the host surface is an interaction of many factors in which environment (Burchfield, 1960 and Courshee, 1967) and host in the form of excreted substances (Wain and Wilkinson, 1945; Kovacs and Kiccuchi, 1964; Dunn *et al.,* 1969; Grover *et al*., 1969; Grover, 1978) play a great role.

Grapes, apples and vegetables like tomato are consumed as a fresh fruit. If the residue level in these produces is more than the MRL, it may be hazardous to the human health and other non-target beneficial organisms. Hence to avoid health hazards, the contaminated produces should be subjected to decontamination treatments to bring down the residue level below the MRL before consumption. Washing the fruits/vegetables with

aqueous solution of common salt and baking soda brought down the residues to a level below MRL and did not affect the physical appearance and taste of the fruits/vegetables. It is absolutely safe for the health of the consumers. Before consumption of any fruit or vegetable it is always advisable to wash thoroughly to eliminate any fungicide residue.

Spray application of recommended dose of fungicides, harvesting the fruits 20 days after last spray and washing with water thoroughly reduce most of the fungicidal residues. Since grapes are consumed as whole fruit, removing the residues to the permissible level is essential as compared to potato or peas which are consumed only after cooking or after peeling (Bhupal Reddy *et al*., 1988). Strict vigilance is therefore needed for need based and more judicious use of fungicides in good varieties of grapes. Therefore from this study it is concluded that hexaconazole at the given recommended dosages by manufacturers are not expected to persist or retain on okra and chilli fruits beyond a week.

CHAPTER VI

SUMMARY

Studies were carried to find out the bioefficacy of hexaconazole 5 SC against powdery mildew disease of okra caused by *E. cichoracearum* DC and chilli powdery mildew disease caused by *L. taurica* (Lev.) Arn., its compatibility with insecticides and biocontrol agents, persistence of hexaconazole, phytotoxic effects, effect on spore germination, effect on phylloplane and rhizosphere microbial population and its harvest time residues in fruits. The results of the experiments are summarised as follows.

1. Hexaconazole sprayed at higher concentration (1500 ml ha^{-1}) recorded the least per cent disease index of powdery mildew disease in okra (1.21 to 1.33 PDI) with highest yield range of 3603.17 to 3865.30 Kg ha^{-1} and the powdery mildew disease in chilli (1.60 to 2.03 PDI) with the highest yield range of 7416.73 to 7782.50 Kg ha^{-1} when compared to their control treatments which recorded a range of 53.77 to 76.77 PDI and 56.57 to 69.17 PDI with the yield of 2859.17 to 3077.83 Kg ha^{-1} and 6240.33 to 6809.10 Kg ha^{-1} respectively in all the three field trials. The green house studies also showed the superiority of hexaconazole in reducing the powdery mildew disease in okra and chilli.

2. The compatibility studies indicated that the hexaconazole (500 ml ha^{-1}) was highly compatible with monocrotophos (1000 ml ha^{-1}) and showed its synergistic action on the pests and powdery mildew disease in okra and chilli. The population of whitefly and leafhopper and incidence of powdery mildew disease were reduced by 77.40 per cent, 82.20 per cent and 75.59 per cent respectively over control in okra. However the

combined treatment of hexaconazole (500 ml ha^{-1}) with mancozeb (1 Kg ha^{-1}) was effective in the control of powdery mildew disease of okra (86.43 per cent reduction over control).

3. In chilli also, the synergistic activity of hexaconazole (500 ml ha^{-1}) with monocrotophos (1000 ml ha^{-1}) was observed against the pests and powdery mildew disease. The population of chilli thrips, aphid damage and the powdery mildew disease were decreased with the per cent damage of 8.73, 16.63 and the per cent disease index of 15.50 PDI as against control which recorded 42.13, 69.73 per cent and 52.53 PDI respectively. Hexaconazole was found to compatible with mancozeb which recorded significantly less per cent disease index of 7.60.

4. The persistence of hexaconazole on okra fruits was observed up to ten days, while it was seven days in chilli fruits. The amount of residues detected were higher for higher concentration. The hexaconazole residues were at below detectable level after 10 and 7 days respectively in okra and chilli crop.

5. No phytotoxic effect of hexaconazole was observed in all the field trials of okra and chilli.

6. Hexaconazole at 2000 ppm recorded the least per cent spore germination of *E. cichoracearum* with 14.93, 8.23 and 3.03 per cent and *L. taurica* with 15.43, 6.10 and 2.00 per cent on 2, 3 and 15 days after treatment respectively as against control, which recorded 78.66, 88.33, 96.33 per cent and 71.00, 81.67 and 86.00 per cent respectively on 2, 3 and 15 days after treatment.

7. The contents of chlorophyll *a, b* and total chlorophyll significantly increased in hexaconazole sprayed okra and chilli leaves at 24 h and 72 h intervals. The total

chlorophyll content of okra leaves increased to 2.15 mg g^{-1} as against the control which showed 1.10 mg g^{-1}. Similarly, the total chlorophyll content of chilli leaves also increased to 2.43 mg g^{-1} as compared to control with 1.49 mg g^{-1} at 72 h after spraying.

8. The hexaconazole (1500 ml ha^{-1}) spray increased the total phenol and soluble protein contents of okra leaves to 540.00, 740.00 mg 100 g^{-1} and 604.00, 613.40 mg 100 g^{-1} as compared with the control treatment which recorded 273.32, 310.00 mg 100 g^{-1} and 480.00, 433.28 mg 100 g^{-1} respectively at 24 h and 72 h after treatment. It also increased the total phenol content in chilli leaves recorded 813.30 and 850.00 mg 100 g^{-1} with the soluble protein content of 545.00 and 628.30 mg 100 g^{-1} when compared to control which showed the total phenol content of 410.00 and 383.41 mg 100 g^{-1} and with the soluble protein content of 374.20 and 370.00 mg 100 g^{-1}.

9. The activity of phenylalanine ammonia lyase (PAL) was high in inoculated okra plants compared to uninoculated control. A significant increase in PAL activity was noticed on 5th day in the application of hexaconazole 1500 ml ha^{-1} compared to other treatments against the powdery mildew pathogen.

10. A significant difference in the activity of phenylalanine ammonia lyase (PAL) was also observed in chilli between treatments, day interval and pathogen inoculation. Maximum PAL activity was observed in the treatment of hexaconazole 1500 ml ha^{-1} on the 5th day compared to standard fungicides against powdery mildew.

11. Significant increases in the activities of peroxidase (PO) and polyphenol oxidase (PPO) were observed upto 72 h in okra plants treated with hexaconazole, compared to other treatments. There was no appreciable change in PO and PPO activity in standard fungicides and control plants.

12. The treatment of hexaconazole 1500 ml ha^{-1} in chilli plants showed enhanced PO and PPO activity in the leaf against the powdery mildew pathogen. A gradual increase in

PO and PPO activity was observed up to 72 h, compared to standard fungicides and untreated control plants.

13. The hexaconazole sprayed chilli leaves showed the induction of peroxidase and polyphenol oxidase isoforms, however there was no difference in its induction.

14. The population of phylloplane fungi and bacteria were found to increase with increase in the concentrations of hexaconazole with a range of 1.72 to 2.69 x 10^3 cfu and 110.00 to 161.70 x 10^5 cfu respectively on 5th day after treatment, however the phylloplane fungal population was high on 0th and 1st day and further it reduced on subsequent day intervals. The phylloplane bacterial population increased with increase in day intervals. The control plants which recorded the phylloplane fungal and bacterial population with a range of 9.00 to 2.60 x 10^3 cfu and with a range of 86.00 to 185.00 x 10^5 cfu with respect to day intervals.

15. The rhizosphere fungal population also reduced with increase in concentrations of hexaconazole with a range of 74.65 to 47.69 x 10^3 cfu (5th day) and it increased with increase in day intervals with a range of 41.67 to 47.69 x 10^3 cfu up to five days after spraying. However, the bacterial population in rhizosphere increased in the hexaconazole treated plants with a range of 162.00 to 261.00 x 10^5 cfu on 5th day after spraying as compared with control plants, which recorded the rhizosphere fungal population of 57.00 x 10^3 cfu and bacterial population of 265.00 x 10^5 cfu on fifth day after spraying.

16. Similarly, the chilli phylloplane fungal population also increased with the increase in concentrations of hexaconazole with a range of 2.00 to 14.00 x 10^3 cfu as compared with control which recorded 24.00 x 10^5 cfu on fifth day after spraying. However the fungal population reduced with increase in day intervals with a range of 17.40 to

14.00 x 10^3 cfu. The phylloplane bacterial population increased with increase in concentrations of hexaconazole with a range of 74.00 to 120.69 x 10^5 cfu on fifth day after spraying and increased with increase in day intervals with a range of 47.00 to 120.69 x 10^5 cfu as compared to control with a population range of 59.00 to 116.00 x 10^5 cfu.

17. The rhizosphere fungal population increased with increase in day intervals (53.00 to 81.00 x 10^3 cfu). However, the population varied between the different concentrations of hexaconazole *viz.,* 500, 750, 1000 and 1500 ml ha^{-1} (93.00, 72.00, 95.65 and 81.00 x 10^3 cfu respectively) when compared to control which recorded 48.00 to 112.00 x 10^3 cfu. The rhizosphere bacterial population recorded no remarkable change between all the treatments of hexaconazole at all the day intervals when compared to control.

18. Hexaconazole at higher concentration of 1.0 ppm showed its superiority in inhibiting the mycelial growth of *Trichoderma viride* (3.1 cm) and *T. harzianum* (3.6 cm), while it was 8.97 cm and 8.93 cm in control plates.

19. Hexaconazole showed maximum inhibition to the mycelial growth of *Alternaria capsici* (2.63 cm) and *Colletotrichum capsici* (2.43 cm) as against the respective control plates which recorded 8.83 cm and 8.50 cm.

20. Hexaconazole residues in the harvested fruits of okra, chilli and in soil were below the detectable level. The mean recovery from the fortified fruits of okra and chilli was 83.40 and 83.51 per cent respectively whereas in soil, the recovery was 82.86 per cent.

Table 13. Regression coefficient of hexaconazole residues in okra

Treat-ment	1st order		1.5^{th} order		2nd order		RF 1st order		RF 1.5^{th} order		RF 2nd order		IPL		Best fit
	R	MR^2	R	MR^2	R	MR^2	R	MR^2	R	MR^2	R	MR^2	R	MR^2	
Trial 1															
T_1	-0.9746	0.9736	0.9829	0.9800	0.9883	0.9412	-0.9661	0.8843	0.9551	0.6939	0.9427	-0.4684	0.9951	0.9496	1.5^{th} order
T_2	-0.9396	0.8687	0.8827	-0.3670	0.8299	-25.3535	-0.8526	0.5792	0.7687	-3.9343	0.7002	-10.2344	0.8037	0.3450	1st order
T_3	-0.9865	0.9658	0.9673	0.8991	0.9361	-0.1378	-0.9376	0.8592	0.8886	0.3624	0.8343	-13.0642	0.8874	0.0873	1st order
T_4	-0.9845	0.9267	0.9289	-9.3842	0.8570	-6.7863	-0.9173	0.4752	0.8120	-1141.01	0.7153	-2.5794	0.8571	0.5320	1st order
Trial 2															
T_1	-0.9737	0.9452	0.9860	0.9359	0.9937	0.9565	-0.9791	0.9042	0.9733	0.8353	0.9632	0.6282	0.9937	-1.1587	2nd order

T_2	-0.9711	0.9443	0.9831	0.9640	0.9919	0.9768	-0.9884	0.9615	0.9834	0.9180	0.9753	0.8053	0.9953	-2.2861	2nd order
T_3	-0.9738	0.9636	0.9814	0.9778	0.9865	0.9847	-0.9720	0.9121	0.9649	0.8391	0.9555	0.6434	0.9855	-1.4740	2nd order
T_4	-0.9740	0.9685	0.9786	0.9743	0.9810	0.9638	-0.9545	0.8550	0.9453	0.7344	0.9344	0.3824	0.9710	-0.8229	1.5th order
Trial 3															
T_1	-0.9372	0.8729	0.9484	0.8892	0.9566	0.9041	-0.9880	0.9862	0.9849	0.9839	0.9792	0.9706	0.9607	-10.3167	RF 1st order
T_2	-0.9621	0.9129	0.9729	0.9272	0.9812	0.9418	-0.9951	0.9912	0.9919	0.9836	0.9863	0.9664	0.9822	-13.1297	RF 1st order
T_3	-0.9840	0.9686	0.9898	0.9788	0.9942	0.9842	-0.9880	0.9689	0.9833	0.9503	0.9774	0.9221	0.9833	-14.3231	2nd order
T_4	-0.9803	0.9583	0.9861	0.9681	0.9909	0.9733	-0.9923	0.9809	0.9888	0.9695	0.9844	0.9522	0.9853	-24.1372	RF 1st order

T_1 – Hexaconazole 500 ml ha^{-1}; T_3 - Hexaconazole 1000 ml ha^{-1}

T_2 - Hexaconazole 750 ml ha^{-1}; T_4 - Hexaconazole 1500 ml ha^{-1}

R - Regression co-efficient; MR^2 – Modified R^2 ; IPL – Inverse Power Law.

Table 14. Intercepts, slope and half life of hexaconazole residues in okra

Treat-ment	A	LL	UL	B	LL	UL	$T_{0.5}$	LL	UL	Waiting Period (days)	Predicted equation
Trial 1											
T_1	0.1513	0.1064	0.1962	0.0350	0.0151	0.0550	1.7868	0.6398	2.9338	15.84	Y=0.1513+0.0350X
T_2	4.1708	3.5046	4.8370	-0.3111	-0.5193	-0.1029	2.2279	0.7371	3.7186	11.17	Y=4.1708-0.3111X
T_3	4.2905	4.0644	4.5166	-0.2324	-0.3031	-0.1618	2.9816	2.0754	3.8879	15.47	Y=4.2905-0.2324X
T_4	4.8922	4.5421	5.2421	-0.3275	-0.4083	-0.2468	2.1160	1.5943	2.6378	12.82	Y=4.8922-0.3275X
Trial 2											
T_1	0.0236	0.0209	0.0263	0.0040	0.0033	0.0046	5.8923	4.7543	7.0302	17.82	Y=0.0236+ 0.0040X
T_2	0.0211	0.0187	0.0234	0.0030	0.0025	0.0036	6.8707	5.4287	8.3128	-	Y=0.0211+0.0030X
T_3	0.0181	0.0152	0.0211	0.0029	0.0022	0.0036	6.1460	4.4157	7.8763	-	Y=0.0181+0.0029X
T_4	0.1241	0.1130	0.1352	0.0088	0.0062	0.0113	5.8298	4.0532	7.6065	18.01	Y=0.1241+0.0088X
Trial 3											
T_1	3.4617	3.3712	3.5522	-0.2324	-0.2828	-0.1820	8.8906	5.0362	12.744 9	-	Y=3.4617-0.2324X
T_2	3.5399	3.4909	3.5889	-0.1987	-0.2260	-0.1715	12.159 9	8.8224	15.497 4	-	Y=3.5399-0.1987X
T_3	0.0303	0.0289	0.0316	-0.0021	0.0017	0.0023	14.615	12.340	16.890	-	Y=0.0303+0.0021X

T_4	3.6105	3.5655	3.6555	-0.1452	-0.1703	-0.1202	5 22.765 2	7 14.912 9	4 30.617 6	-	Y=3.6105-0.1452X

T_1 – Hexaconazole 500 ml ha^{-1};

T_3 - Hexaconazole 1000 ml ha^{-1}

T_2 - Hexaconazole 750 ml ha^{-1};

T_4 - Hexaconazole 1500 ml ha^{-1}

A – intercept; LL – Lower Limit ; UL – Upper Limit; B – Slope ; $T_{0.5}$ – Half life

Table 17. Regression coefficient of hexaconazole residues in chilli

Treatment	1st order		1.5 order		2nd order		RF 1st order		RF 1.5 order		RF 2nd order		IPL		Best fit
	R	MR^2	R	MR^2	R	MR^2	R	MR^2	R	MR^2	R	MR^2	R	MR^2	
Trial 1															
T_1	-0.9760	0.9598	0.9428	0.7686	0.9021	-1.8925	-0.9011	0.7359	0.8446	-0.0154	0.7867	-26.8425	0.8836	0.3264	1st order
T_2	-0.9207	0.8471	0.8850	0.7128	0.8458	-1.8418	-0.8623	0.8001	0.7953	0.2617	0.7344	-25.7318	0.7331	-0.8765	1st order
T_3	-0.9358	0.8639	0.9157	0.8823	0.8817	0.6201	-0.9161	0.9233	0.8585	0.7381	0.7946	-2.7108	0.7789	-0.9816	RF 1st order
T_4	-0.8921	0.7192	0.9250	0.7368	0.9486	0.7589	-0.9734	0.9467	0.9799	0.9706	0.9757	0.9894	0.9261	-6.4703	RF 2nd order
Trial 2															
T_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_3	-0.9910	0.9588	0.9713	0.7287	0.9438	-3.117	-0.915	0.6746	0.8743	-0.168	0.8306	-42.630	0.9589	0.9266	1st order

						4	0			9		0			
T_4	-0.9593	0.9154	0.9522	0.7896	0.9457	-0.2378	-0.8992	0.6605	0.8761	-0.0821	0.8567	-27.7855	0.8919	0.4281	1st order
Trial 3															
T_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T_2	-0.9999	0.9999	0.9992	0.9970	0.9965	0.9815	-0.9631	0.9199	0.9494	0.8733	0.9350	0.7946	-	-	1st order
T_3	-0.9879	0.9679	0.9958	0.9857	0.9995	0.9979	-0.9923	0.9852	0.9822	0.9603	0.9687	0.9058	-	-	2nd order
T_4	-0.9931	0.9775	0.9883	0.9476	0.9831	0.8889	-0.9223	0.8144	0.9081	0.7333	0.8946	0.5965	-	-	1st order

T_1 – Hexaconazole 500 ml ha^{-1}; T_3 - Hexaconazole 1000 ml ha^{-1}

T_2 - Hexaconazole 750 ml ha^{-1}; T_4 - Hexaconazole 1500 ml ha^{-1}

R - Regression co-efficient; MR^2 – Modified R^2 ; IPL – Inverse Power Law.

Table 18. Intercepts, slope and half life of hexaconazole residues in chilli

Treat-ment	A	LL	UL	B	LL	UL	$T_{0.5}$	LL	UL	Waiting period (days)	Predicted equation
Trial 1											
T_1	2.9107	2.6051	3.2163	-0.2334	-0.3288	-0.1379	2.9698	1.7547	4.1849	9.50	Y=2.9107-0.2334X
T_2	3.2367	2.6802	3.7933	-0.2233	-0.3972	-0.0494	3.1031	0.6870	5.5193	11.38	Y=3.2367-0.2233X
T_3	3.6136	2.9350	4.2921	-0.5574	-1.0030	-0.1118	1.5459	-0.9254	4.0174	-	Y=3.6136-0.5574X
T_4	0.0247	0.0121	0.0373	0.0200	0.0118	0.0283	1.2330	2.8472	3.8110	-	Y=0.0247+0.0200 X
Trial 2											
T_1	-	-	-	-	-	-	-	-	-	-	-
T_2	-	-	-	-	-	-	-	-	-	-	-
T_3	3.2826	2.9481	3.6171	-0.3631	-0.5117	-0.2144	1.9088	1.1273	2.6903	7.13	Y=3.2826-0.3631X
T_4	3.5033	3.0623	3.9443	-0.2551	-0.3929	-0.1173	2.7169	1.2492	4.1846	11.01	Y=3.5033-0.2551X

Trial 3											
T_1	-	-	-	-	-	-	-	-	-	-	-
T_2	2.5629	2.5356	2.5903	-0.2574	-0.2779	-0.2368	2.6928	2.4781	2.9076	7.26	Y=2.5629-0.2574X
T_3	0.0508	0.0387	0.0629	0.0248	0.0158	0.0339	2.0442	1.1541	2.9342	18.06	Y=0.0508+0.0248 X
T_4	3.6212	3.0680	4.1744	-0.2765	-0.6915	0.1383	2.5060	-1.2532	6.2652	10.58	Y=3.6212-0.2765X

T_1 – Hexaconazole 500 ml ha^{-1}; T_3 - Hexaconazole 1000 ml ha^{-1}

T_2 - Hexaconazole 750 ml ha^{-1}; T_4 - Hexaconazole 1500 ml ha^{-1}

A – intercept; LL – Lower Limit ; UL – Upper Limit; B – Slope ; $T_{0.5}$ – Half life

Table 19. Evaluation of phytotoxicity of hexaconazole in okra

Treatment	Phytotoxicity parameters (based on grade 1-10 scale)																	
	Leaf injury on tips			Leaf injury on surface			Vein clearing			Necrosis			Epinasty			Hyponasty		
	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III
500 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
750 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
1000 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
1500 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	1	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Control	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP

NP – No phytotoxicity

1 = less than 1 per cent level of injury

Table 20. Evaluation of phytotoxicity of hexaconazole in chilli

Treatment	Phytotoxicity parameters (based on grade 1-10 scale)																	
	Leaf injury on tips			Leaf injury on surface			Vein clearing			Necrosis			Epinasty			Hyponasty		
	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III
500 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
750 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
1000 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
1500 ml ha^{-1}	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
Control	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP

NP – No phytotoxicity

1 = less than 1 per cent level of injury

Table 25. Effect of fungicides on chlorophyll content of okra leaves

Treatment	Chlorophyll content (mg g^{-1})*					
	24 h after spraying			72 h after spraying		
	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Hexaconazole 500 ml ha^{-1}	1.01^{c}	0.40^{d}	1.40^{e}	1.15^{d}	0.51^{ef}	1.52^{c}
Hexaconazole 750 ml ha^{-1}	1.04^{c}	0.47^{bc}	1.46^{d}	1.26^{c}	0.68^{c}	1.75^{c}
Hexaconazole 1000 ml ha^{-1}	1.12^{ab}	0.49^{b}	1.59^{b}	1.41^{b}	0.73^{b}	1.92^{b}
Hexaconazole 1500 ml ha^{-1}	1.17^{a}	0.58^{a}	1.74^{a}	1.55^{a}	0.89^{a}	2.15^{a}
Mancozeb 1Kg ha^{-1}	1.11^{b}	0.43^{cd}	1.54^{bc}	1.25^{c}	0.61^{d}	1.72^{cd}
Propiconazole 1000 ml ha^{-1}	1.09^{b}	0.42^{cd}	1.51^{cd}	1.23^{c}	0.54^{e}	1.68^{d}

Dinocap 750 ml ha^{-1}	0.95^{d}	0.33^{e}	1.28^{f}	1.16^{d}	0.47^{f}	1.53^{e}
Wettable sulphur 1500 ml ha^{-1}	0.87^{e}	0.32^{e}	1.20^{g}	1.07^{e}	0.38^{g}	1.46^{f}
Control (sterile water)	0.65^{f}	0.24^{f}	0.95^{k}	0.81^{f}	0.31^{h}	1.10^{g}

CD for t x h means = 0.04

* Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 26. Effect of fungicides on chlorophyll content of chilli leaves

Treatment	Chlorophyll content (mg g^{-1})*					
	24 h after spraying			72 h after spraying		
	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Hexaconazole 500 ml ha^{-1}	1.42^{d}	0.45^{d}	1.90^{d}	1.60^{d}	0.51^{d}	2.10^{d}
Hexaconazole 750 ml ha^{-1}	1.54^{c}	0.52^{c}	2.05^{c}	1.72^{c}	0.64^{c}	2.28^{c}
Hexaconazole 1000 ml ha^{-1}	1.68^{b}	0.61^{b}	2.12^{b}	1.84^{b}	0.78^{b}	2.35^{b}
Hexaconazole 1500 ml ha^{-1}	1.83^{a}	0.74^{a}	2.28^{a}	1.93^{a}	0.97^{a}	2.43^{a}
Mancozeb 1Kg ha^{-1}	1.41^{d}	0.43^{de}	1.82^{e}	1.58^{d}	0.49^{d}	2.08^{d}
Propiconazole 750 ml ha^{-1}	1.38^{d}	0.38^{ef}	1.78^{e}	1.52^{e}	0.47^{d}	2.01^{e}
Tridemorph 1000 ml ha^{-1}	1.25^{e}	0.34^{fg}	1.65^{f}	1.41^{f}	0.41^{e}	1.76^{f}

Wettable sulphur 1000 ml ha^{-1}	1.12^{f}	0.29^{g}	1.47^{g}	1.20^{g}	0.36^{e}	1.62^{g}
Control (sterile water)	0.87^{g}	0.20^{h}	1.33^{h}	1.08^{h}	0.29^{f}	1.49^{h}

CD for t x h means =
0.05

* Mean of three replications.

In a column, mean followed by a common letter are not significantly different at 5% level by DMRT.

Table 29. Effect of hexaconazole 5 SC on induction of phenylalanine ammonialyase activity in okra

Treatment	PAL enzyme activity (m.mol equ. min^{-1} ml^{-1})*							
	Uninoculated plants				Inoculated plants			
	Days after treatment							
	0	1	3	5	0	1	3	5
Hexaconazole 500 ml ha^{-1}	1.32[e]	1.34[e]	1.45[d]	1.48[d]	1.40[c]	1.38[e]	1.39[f]	1.49[d]
Hexaconazole 750 ml ha^{-1}	1.41[d]	1.45[d]	1.69[c]	1.83[c]	1.54[b]	1.60[d]	1.82[d]	2.11[c]
Hexaconazole 1000 ml ha^{-1}	1.57[b]	1.60[c]	1.89[b]	2.07[b]	1.80[a]	1.98[b]	2.30[b]	2.36[b]
Hexaconazole 1500 ml ha^{-1}	1.67[a]	1.71[a]	2.15[a]	2.23[a]	1.85[a]	2.13[a]	2.50[a]	2.75[a]
Mancozeb 1Kg ha^{-1}	1.50[c]	1.65[b]	1.96[b]	2.05[b]	1.60[b]	1.78[c]	1.96[c]	2.08[c]
Propiconazole 1000 ml ha^{-1}	1.37[d]	1.38[e]	1.51[d]	1.44[de]	1.41[c]	1.53[d]	1.49[e]	1.50[d]
Dinocap 1000 ml ha^{-1}	1.13[f]	1.30[f]	1.44[d]	1.48[d]	1.16[d]	1.37[e]	1.42[f]	1.47[d]

Wettable Sulphur 1500 ml ha^{-1}	1.11^{fg}	1.14^{g}	1.20^{e}	1.35^{ef}	1.10^{d}	1.23^{f}	1.19^{g}	1.31^{e}
Control (Sterile water)	1.08^{g}	1.11^{g}	1.07^{e}	1.30^{f}	1.14^{d}	1.16^{g}	1.15^{g}	1.27^{e}
CD			0.08				0.06	

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 30. Effect of hexaconazole 5 SC on the induction of phenylalanine ammonia lyase activity in chilli

Treatment	PAL enzyme activity (m.mol min^{-1} ml^{-1})*							
	Uninoculated plants				Inoculated plants			
	Days after treatment							
	0	1	3	5	0	1	3	5
Hexaconazole 500 ml ha^{-1}	1.35^{bcd}	1.39^{bcd}	1.41^{c}	1.44^{c}	1.39^{bcd}	1.42^{c}	1.44^{d}	1.48^{cd}
Hexaconazole 750 ml ha^{-1}	1.41^{bc}	1.48^{bc}	1.58^{b}	1.63^{b}	1.44^{bc}	1.54^{b}	1.60^{c}	1.70^{bc}
Hexaconazole 1000 ml ha^{-1}	1.47^{ab}	1.54^{ab}	1.66^{ab}	1.76^{ab}	1.50^{b}	1.61^{b}	1.76^{b}	1.89^{b}
Hexaconazole 1500 ml ha^{-1}	1.56^{a}	1.65^{a}	1.78^{a}	1.91^{a}	1.83^{a}	2.07^{a}	2.24^{a}	2.72^{a}
Mancozeb 1Kg ha^{-1}	1.26^{cd}	1.42^{bcd}	1.40^{c}	1.41^{c}	1.31^{cde}	1.36^{cd}	1.43^{d}	1.45^{cd}
Propiconazole 1000 ml ha^{-1}	1.24^{d}	1.29^{d}	1.31^{c}	1.34^{c}	1.24^{e}	1.27^{de}	1.31^{ef}	1.33^{d}

Tridemorph 1000 ml ha^{-1}	1.27^{cd}	1.32^{d}	1.32^{c}	1.32^{c}	1.25^{e}	1.26^{de}	1.27^{ef}	1.30^{d}
Wettable Sulphur 1500 ml ha^{-1}	1.30^{cd}	1.28^{d}	1.29^{c}	1.33^{c}	1.22^{e}	1.20^{e}	1.24^{f}	1.25^{d}
Control	1.26^{cd}	1.35^{cd}	1.35^{c}	1.28^{c}	1.30^{de}	1.35^{cd}	1.36^{de}	1.39^{d}
CD	0.15				0.17			

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 31. Effect of hexaconazole 5SC on induction of peroxidase and polyphenol oxidase activity in okra plants

Treatment	Peroxidase enzyme activity* expressed in changes in absorb. gm^{-1} min^{-1}		Polyphenol oxidase enzyme activity* expressed in changes in absorb. gm^{-1} min^{-1}	
	Hours after treatment			
	24h	72h	24h	72h
Hexaconazole 500 ml ha^{-1}	0.47^{cd}	0.72^{d}	0.29^{b}	0.65^{cd}
Hexaconazole 750 ml ha^{-1}	0.63^{c}	1.45^{c}	0.34^{b}	0.84^{bc}
Hexaconazole 1000 ml ha^{-1}	1.13^{b}	1.81^{b}	0.50^{ab}	1.00^{b}
Hexaconazole 1500 ml ha^{-1}	1.37^{a}	2.54^{a}	0.70^{a}	1.50^{a}
Mancozeb 1Kg ha^{-1}	0.39^{de}	0.72^{d}	0.32^{b}	0.80^{bc}
Propiconazole 1000 ml ha^{-1}	0.35^{de}	0.61^{de}	0.26^{b}	0.60^{cd}
Dinocap 1000 ml ha^{-1}	0.29^{de}	0.55^{e}	0.23^{b}	0.59^{cd}
Wettable sulphur 1500 ml ha^{-1}	0.26^{de}	0.43^{e}	0.21^{b}	0.56^{cd}
Control (untreated)	0.20^{e}	0.54^{de}	0.20^{b}	0.45^{d}
CD for t x h means	0.20		0.26	

* Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 32. Effect of hexaconazole 5 SC on induction of peroxidase and polyphenol oxidase activity in chilli plants

Treatment	Peroxidase enzyme activity* expressed in changes in absorb. gm^{-1} min^{-1}		Polyphenol oxidase enzyme activity* expressed in changes in absorb. gm^{-1} min^{-1}	
	Hours after treatment			
	24h	72h	24h	72h
Hexaconazole 500 ml ha^{-1}	0.33^{cd}	0.56^{d}	0.32^{b}	0.45^{cd}
Hexaconazole 750 ml ha^{-1}	0.38^{c}	0.72^{c}	0.54^{a}	0.62^{bc}
Hexaconazole 1000 ml ha^{-1}	0.53^{b}	1.02^{b}	0.58^{a}	0.81^{ab}
Hexaconazole 1500 ml ha^{-1}	0.71^{a}	1.25^{a}	0.73^{a}	0.99^{a}
Mancozeb 1Kg ha^{-1}	0.29^{d}	0.49^{e}	0.33^{b}	0.54^{cd}
Propiconazole 1000 ml ha^{-1}	0.27^{d}	0.45^{ef}	0.22^{b}	0.47^{cd}
Tridemorph 1000 ml ha^{-1}	0.25^{de}	0.41^{f}	0.20^{b}	0.40^{d}
Wettable sulphur 1500 ml ha^{-1}	0.19^{ef}	0.39^{f}	0.18^{b}	0.38^{d}
Control (untreated)	0.16^{f}	0.36^{f}	0.15^{b}	0.35^{d}
CD for t x h means	0.07		0.19	

* Mean of three replications

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 33. Effect of hexaconazole 5SC on the population of phylloplane microflora in okra

Treatment	Mean microbial population of phylloplane*							
	No. of fungal colonies (cfu ml^{-1} x 10^3)				No. of bacterial colonies (cfu ml^{-1} x 10^5)			
	Days after spraying							
	0	1	3	5	0	1	3	5
Hexaconazole 500 ml ha^{-1}	4.0^d	2.0^c	1.0^d	2.0^b	43.0^d	59.0^c	72.0^f	110.0^f
Hexaconazole 750 ml ha^{-1}	6.0^c	3.0^c	2.0^c	1.0^c	57.0^b	51.0^d	93.0^e	125.0^d
Hexaconazole 1000 ml ha^{-1}	11.0^b	7.0^b	3.0^b	1.0^a	53.0^c	78.0^b	107.0^c	140.0^c
Hexaconazole 1500 ml ha^{-1}	15.0^a	9.0^a	3.0^a	4.0^a	60.0^b	81.0^b	120.0^b	162.0^b
Mancozeb 1Kg ha^{-1}	7.0^c	1.0^d	1.0^d	1.0^c	51.0^c	45.0^e	98.0^d	116.0^e
Control (Sterile water)	9.0^b	6.0^b	4.0^a	3.0^a	86.0^a	103.0^a	143.0^a	185.0^a

CD for fungal t x d means = 1.00

CD for bacterial t x d means = 3.57

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 34. Effect of hexaconazole 5SC on the population of rhizosphere microflora in okra

Treatment	Mean microbial population of phylloplane*							
	No. of fungal colonies (cfu ml^{-1} x 10^3)				No. of bacterial colonies (cfu ml^{-1} x 10^5)			
	Days after spraying							
	0	1	3	5	0	1	3	5
Hexaconazole 500 ml ha^{-1}	58.0^{a}	67.0^{a}	63.0^{b}	75.0^{a}	210.0^{b}	188.0^{d}	185.0^{e}	162.0^{e}
Hexaconazole 750 ml ha^{-1}	34.0^{e}	31.0^{d}	40.0^{e}	51.0^{d}	207.0^{bc}	227.0^{b}	196.0^{d}	261.0^{b}
Hexaconazole 1000 ml ha^{-1}	39.0^{cd}	48.0^{b}	53.0^{e}	69.0^{b}	223.0^{a}	217.0^{c}	233.0^{c}	208.0^{d}
Hexaconazole 1500 ml ha^{-1}	42.0^{c}	37.0^{c}	34.0^{f}	48.0^{d}	218.0^{a}	231.0^{b}	197.0^{d}	159.0^{e}
Mancozeb 1Kg ha^{-1}	36.0^{de}	33.0^{d}	45.0^{d}	41.0^{e}	201.0^{c}	230.0^{a}	276.0^{a}	225.0^{c}
Control (Sterile water)	50.0^{b}	64.0^{a}	69.0^{a}	57.0^{c}	175.0^{d}	185.0^{d}	265.0^{b}	282.0^{a}

CD for fungal t x d means = 4.23

CD for bacterial t x d means = 6.13

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 35. Effect of hexaconazole 5SC on the population of phylloplane microflora in chilli

Treatment	Mean microbial population of phylloplane*							
	No. of fungal colonies (cfu ml^{-1} x 10^3)				No. of bacterial colonies (cfu ml^{-1} x 10^5)			
	Days after spraying							
	0	1	3	5	0	1	3	5
Hexaconazole 500 ml ha^{-1}	8.0^{d}	7.0^{c}	5.0^{d}	2.0^{d}	28.0^{d}	39.0^{d}	58.0^{e}	74.0^{f}
Hexaconazole 750 ml ha^{-1}	11.0^{cd}	9.0^{c}	7.0^{d}	3.0^{d}	27.0^{d}	36.0^{d}	73.0^{d}	97.0^{d}
Hexaconazole 1000 ml ha^{-1}	13.0^{bc}	14.0^{b}	17.0^{b}	8.0^{c}	35.0^{c}	47.0^{c}	91.0^{c}	112.0^{c}
Hexaconazole 1500 ml ha^{-1}	18.0^{a}	17.0^{ab}	24.0^{a}	14.0^{b}	47.0^{b}	55.0^{b}	110.0^{a}	121.0^{a}
Mancozeb 1K g ha^{-1}	9.0^{d}	7.0^{c}	12.0^{c}	6.0^{c}	31.0^{d}	29.0^{e}	56.0^{e}	85.0^{e}
Control (Sterile water)	15.0^{ab}	18.0^{a}	21.0^{a}	24.0^{a}	59.0^{a}	71.0^{a}	95.0^{b}	116.0^{b}

CD for fungal t x d means = 3.31

CD for bacterial t x d means = 3.86

* Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 36. Effect of hexaconazole 5 SC on the population of rhizosphere microflora in chilli

Treatment	Mean microbial population in chilli rhizosphere							
	No. of fungal colonies (cfu ml^{-1}* x 10^3)				No. of bacterial colonies (cfu ml^{-1}* x 10^5)			
	Days after spraying							
	0	1	3	5	0	1	3	5
Hexaconazole 500 ml ha^{-1}	49.0^{ab}	56.0^{c}	77.0^{c}	93.0^{b}	178.0^{d}	193.0^{d}	221.0^{d}	198.0^{f}
Hexaconazole 750 ml ha^{-1}	38.0^{c}	52.0^{c}	51.0^{e}	72.0^{d}	181.0^{d}	175.0^{f}	209.0^{e}	231.0^{c}
Hexaconazole 1000 ml ha^{-1}	47.0^{b}	64.0^{b}	73.0^{c}	96.0^{b}	169.0^{e}	179.0^{e}	194.0^{f}	211.0^{e}
Hexaconazole 1500 ml ha^{-1}	53.0^{a}	79.0^{a}	87.0^{b}	81.0^{c}	195.0^{c}	223.0^{c}	235.0^{c}	217.0^{d}
Mancozeb 1Kg ha^{-1}	33.0^{c}	46.0^{d}	65.0^{d}	85.0^{c}	215.0^{b}	238.0^{b}	255.0^{b}	248.0^{b}
Control (Sterile water)	48.0^{b}	67.0^{e}	97.0^{a}	112.0^{a}	227.0^{a}	260.0^{a}	277.0^{a}	296.0^{a}

CD for fungal t x d means = 4.14

CD for bacterial t x d means = 3.93

*Mean of three replications

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 3. Bioefficacy of hexaconazole 5 SC against okra powdery mildew under field condition (Oct.-Dec. 1999)

Treatment	Per cent Disease Index*		Per cent decrease over control	Yield * Kg ha^{-1}	Per cent increase over control
	Before spray	After third spray			
Hexaconazole 500 ml ha^{-1}	26.83[e] (31.20)	7.54[d] (15.93)	85.97	3591.83[d]	16.76
Hexaconazole 750 ml ha^{-1}	27.43[c] (31.58)	3.82[c] (11.26)	92.90	3726.87[c]	21.15
Hexaconazole 1000 ml ha^{-1}	28.63[b] (32.35)	2.61[b] (9.3)	95.14	3799.33[b]	23.51
Hexaconazole 1500 ml ha^{-1}	29.33[a] (32.79)	1.21[a] (6.3)	97.70	3865.30[a]	25.65
Mancozeb 1Kg ha^{-1}	25.63[f] (30.42)	10.73[e] (19.12)	80.04	3548.50[e]	15.35
Propiconazole 1000 ml ha^{-1}	24.80[g] (29.87)	12.07[f] (20.32)	77.55	3407.67[f]	10.78
Dinocap 1000 ml ha^{-1}	24.10[h] (29.40)	14.57[g] (22.43)	72.90	3236.00[g]	5.19
Wettable sulphur 1500 ml ha^{-1}	23.60[i] (29.06)	15.36[h] (23.07)	71.43	3198.17[b]	3.96
Control (untreated)	27.13[d] (31.39)	53.77[i] (47.16)	-	3076.18[i]	-
CD		0.57		12.86	

* Mean of three replications
Values in the parentheses are arcsine transformed values
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 4. Bioefficacy of hexaconazole 5 SC against okra powdery mildew under field conditions (Dec.1999-Feb. 2000)

Treatment	Per cent Disease Index*		Per cent decrease over control	Yield * Kg ha^{-1}	Per cent increase over control
	Before spray	After third spray			
Hexaconazole 500 ml ha^{-1}	27.00^{b} (31.29)	8.84^{c} (17.32)	88.45	3355.00^{d}	17.34
Hexaconazole 750 ml ha^{-1}	27.77^{b} (31.80)	6.58^{f} (14.85)	91.43	3477.93^{c}	21.64
Hexaconazole 1000 ml ha^{-1}	29.80^{a} (33.08)	4.23^{g} (11.87)	94.50	3550.50^{b}	24.18
Hexaconazole 1500 ml ha^{-1}	30.33^{a} (33.41)	1.27^{h} (6.46)	98.34	3603.17^{a}	26.02
Mancozeb 1Kg ha^{-1}	26.70^{bc} (31.10)	9.70^{e} (18.15)	87.40	3307.27^{e}	15.67
Propiconazole 1000 ml ha^{-1}	26.60^{bc} (31.04)	13.33^{d} (21.42)	82.64	3177.57^{f}	11.14
Dinocap 1000 ml ha^{-1}	25.10^{cd} (30.06)	15.43^{c} (23.13)	79.90	3018.93^{g}	5.59
Wettable sulphur 1500 ml ha^{-1}	24.57^{d} (29.70)	18.27^{b} (25.30)	76.20	3007.00^{h}	5.17
Control	26.70^{bc} (31.10)	76.77^{a} (61.2)	-	2859.17^{I}	-
CD		1.08		8.26	

* Mean of three replications

Values in the parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 5. Bioefficacy of hexaconazole 5 SC against okra powdery mildew under field conditions (Oct.1999-Dec. 2000)

Treatment	Per cent Disease Index*		Per cent decrease over control	Yield * Kg ha^{-1}	Per cent increase over control
	Before spray	After third spray			
Hexaconazole 500 ml ha^{-1}	23.63^{c} (29.09)	6.43^{d} (14.69)	89.50	3593.33^{d}	16.75
Hexaconazole 750 ml ha^{-1}	25.30^{b} (30.19)	5.13^{c} (13.09)	91.62	3599.83^{c}	16.96
Hexaconazole 1000 ml ha^{-1}	25.60^{b} (30.39)	2.97^{b} (9.92)	95.15	3677.30^{b}	19.48
Hexaconazole 1500 ml ha^{-1}	27.13^{a} (31.40)	1.33^{a} (6.63)	97.80	3782.03^{a}	22.88
Mancozeb 1Kg ha^{-1}	22.43^{d} (28.27)	8.73^{e} (17.19)	85.73	3423.17^{e}	11.22
Propiconazole 1000 ml ha^{-1}	21.50^{e} (27.62)	10.47^{f} (18.87)	82.89	3317.60^{f}	7.79
Dinocap 1000 ml ha^{-1}	20.43^{f} (26.87)	12.00^{g} (20.27)	80.39	3216.83^{g}	4.52
Wettable sulphur 1500 ml ha^{-1}	19.81^{g} (26.39)	14.87^{h} (22.68)	75.70	3184.00^{h}	3.45
Control	25.60^{b} (30.39)	61.20^{i} (51.47)	-	3077.83^{i}	-
CD		0.45		3.71	

* Mean of three replications
Values in the parentheses are arcsine transformed values
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 5a. Pooled analysis on bioefficacy of hexaconazole 5 SC against okra powdery mildew for three trials (Oct.-Dec. 1999 ; Dec.1999-Feb. 2000 and Oct.-Dec. 2000)

Treatments	Per cent disease index*			Mean*	Per cent reduction over control	Yield (Kg ha^{-1})	C:B ratio
	Trials						
	1	2	3				
Hexaconazole 500 ml ha^{-1}	7.53^{d}	8.87^{d}	6.43^{d}	7.61	88.09	3513.39	1:1.38
Hexaconazole 750 ml ha^{-1}	3.81^{c}	6.57^{c}	5.13^{c}	5.17	91.91	3601.54	1:1.42
Hexaconazole 1000 ml ha^{-1}	2.61^{b}	4.23^{b}	2.98^{b}	3.27	94.88	3675.71	1:1.45
Hexaconazole 1500 ml ha^{-1}	1.20^{a}	1.27^{a}	1.3a	1.26	98.03	3750.17	1:1.49
Mancozeb 1Kg ha^{-1}	10.73^{e}	9.70^{d}	8.73^{e}	9.72	84.79	3426.31	1:1.37
Propiconazole 1000 ml ha^{-1}	12.07^{f}	13.33^{e}	10.47^{f}	11.95	81.30	3300.95	1:1.35
Dinocap 1000 ml ha^{-1}	14.56^{g}	15.43^{f}	12.00^{g}	14.00	78.09	3157.25	1:1.31
Wettable sulphur 1500 ml ha^{-1}	15.36^{g}	18.27^{g}	14.87^{h}	16.17	74.69	3129.72	1:1.27
Control	5.77^{h}	76.77^{h}	61.20^{i}	63.91	-	3004.39	-
CD				1.25			

* Mean of pooled replications
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 6. Bioefficacy of hexaconazole 5SC against chilli powdery mildew under field conditions (Oct. 1999-Mar. 2000)

Treatment	Per cent Disease Index*		Per cent decrease over control	Yield * Kg ha^{-1}	Per cent increase over control
	Before spray	After third spray			
Hexaconazole 500 ml ha^{-1}	20.53^{e} (26.94)	5.90^{d} (14.05)	89.57	7369.43^{d}	8.23
Hexaconazole 750 ml ha^{-1}	21.43^{d} (27.58)	4.00^{c} (11.53)	92.93	7452.53^{c}	9.45
Hexaconazole 1000 ml ha^{-1}	22.70^{c} (28.45)	3.30^{b} (10.46)	94.17	7575.67^{b}	11.26
Hexaconazole 1500 ml ha^{-1}	23.71^{a} (29.13)	1.60^{a} (7.26)	97.20	7674.73^{a}	12.71
Mancozeb 1 Kg ha^{-1}	19.23^{f} (26.01)	7.30^{e} (15.67)	87.10	7333.17^{d}	7.70
Propiconazole 1000 ml ha^{-1}	18.53^{g} (25.50)	9.00^{f} (17.45)	84.09	7242.00^{e}	6.36
Tridemorph 1000 ml ha^{-1}	17.98^{h} (25.08)	9.80^{g} (18.24)	82.68	7097.67^{f}	4.24
Wettable sulphur 1500 ml ha^{-1}	17.37^{i} (24.63)	11.00^{h} (19.37)	80.55	7047.33^{f}	3.50
Control	23.40^{b} (28.93)	56.57^{i} (48.78)	-	6809.10^{g}	-
CD		0.61		57.74	

* Mean of three replications
Values in the parentheses are arcsine transformed values
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 7. Bioefficacy of hexaconazole 5SC against chilli powdery mildew under field conditions (Aug. 2000-Jan. 2001)

Treatment	Per cent Disease Index*		Per cent decrease over control	Yield * Kg ha^{-1}	Per cent increase over control
	Before spray	After third spray			
Hexaconazole 500 ml ha^{-1}	25.70^{e} (30.45)	7.03^{d} (15.38)	88.90	7235.53^{d}	7.79
Hexaconazole 750 ml ha^{-1}	27.10^{c} (31.36)	5.83^{c} (13.97)	90.79	7396.17^{c}	10.18
Hexaconazole 1000 ml ha^{-1}	28.70^{b} (32.38)	4.20^{b} (11.82)	93.36	7623.00^{b}	13.56
Hexaconazole 1500 ml ha^{-1}	30.00^{a} (33.20)	2.03^{a} (8.19)	96.79	7782.50^{a}	15.94
Mancozeb 1Kg ha^{-1}	25.03^{f} (30.01)	8.37^{e} (16.80)	86.78	7195.33^{e}	7.19
Propiconazole 1000 ml ha^{-1}	23.83^{g} (29.21)	10.43^{f} (18.80)	83.52	7157.50^{f}	6.63
Tridemorph 1000 ml ha^{-1}	25.20^{f} (30.12)	12.57^{g} (20.76)	80.14	7118.00^{g}	6.04
Wettable sulphur 1500 ml ha^{-1}	26.17^{d} (30.76)	15.10^{h} (22.86)	76.14	7106.33^{h}	5.87
Control	29.90^{a} (33.14)	63.30^{i} (52.73)	-	6712.53^{I}	-
CD		0.42		6.61	

* Mean of three replications
Values in the parentheses are arcsine transformed values
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 8. Bioefficacy of hexaconazole 5 SC against chilli powdery mildew under field conditions (Aug. 2001-Jan. 2002)

Treatment	Per cent Disease Index*		Per cent decrease over control	Yield * Kg ha^{-1}	Per cent increase over control
	Before spray	After third spray			
Hexaconazole 500 ml ha^{-1}	21.03^{h} (27.29)	4.90^{d} (12.79)	92.90	6979.37^{f}	11.84
Hexaconazole 750 ml ha^{-1}	22.97^{f} (28.63)	4.10^{c} (11.68)	94.07	7184.21^{c}	15.12
Hexaconazole 1000 ml ha^{-1}	23.83^{c} (29.22)	3.60^{b} (10.94)	94.79	7246.55^{b}	16.14
Hexaconazole 1500 ml ha^{-1}	26.47^{a} (30.95)	1.73^{a} (7.56)	97.50	7416.73^{a}	18.85
Mancozeb 1Kg ha^{-1}	23.73^{c} (29.10)	6.13^{e} (14.30)	91.14	7070.98^{d}	13.31
Propiconazole 1000 ml ha^{-1}	23.27^{e} (28.80)	7.80^{f} (16.22)	88.72	7007.12^{e}	12.29
Tridemorph 1000 ml ha^{-1}	21.338^{g} (27.50)	9.30^{g} (17.80)	86.55	6770.51^{g}	8.50
Wettable sulphur 1500 ml ha^{-1}	23.47^{d} (28.96)	12.10^{h} (20.35)	82.51	6485.50^{h}	3.93
Control	25.10^{b} (30.06)	69.17^{i} (56.28)	-	6240.33^{i}	-
CD		0.51		6.15	

* Mean of three replications

Values in the parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 8a. Pooled analysis on bioefficacy of hexaconazole 5 SC against chilli powdery mildew for three trials (Oct. 1999-Mar. 2000; Aug. 2000-Jan. 2001 and Aug. 2001-Jan. 2002)

Treatments	Per cent disease index*			Mean*	Per cent reduction over control	Yield (Kg ha^{-1})	C:B ratio
	Trials						
	1	2	3				
Hexaconazole 500 ml ha^{-1}	5.90 ^{c}	7.03 ^{d}	4.90 ^{cd}	5.94	90.57	7194.78	1:1.55
Hexaconazole 750 ml ha^{-1}	4.00 ^{b}	5.83 ^{c}	4.10 ^{c}	4.64	92.64	7344.30	1:1.60
Hexaconazole 1000 ml ha^{-1}	3.30 ^{b}	4.20 ^{b}	3.60 ^{b}	3.70	91.13	7481.74	1:1.65
Hexaconazole 1500 ml ha^{-1}	1.6a	2.03 ^{a}	1.7^{a}	1.79	97.15	7624.65	1:1.68
Mancozeb 1Kg ha^{-1}	7.30 ^{d}	8.37 ^{e}	6.13 ^{d}	7.07	88.46	7199.83	1:1.53
Propiconazole 1000 ml ha^{-1}	9.00 ^{e}	10.43 ^{f}	7.80 ^{e}	9.07	85.61	7135.54	1:1.51
Tridemorph 1000 ml ha^{-1}	9.80 ^{e}	12.57 ^{g}	9.33 ^{f}	10.57	83.23	6995.39	1:1.47
Wettable sulphur 1500 ml ha^{-1}	11.00 ^{f}	15.10 ^{h}	12.10 ^{g}	12.73	79.80	6879.72	1:1.43
Control	56.57 ^{g}	63.33 ^{i}	69.17 ^{h}	68.02	-	6587.32	-
CD				1.32			

* Mean of pooled replications
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 9. Compatibility of hexaconazole 5SC with insecticide for the management of pests and powdery mildew disease in okra

Treatments	White fly* (Mean population)* in numbers	Leaf hopper (Mean population)# in numbers	Powdery mildew[O] (Per cent Disease Index)[O]
Hexaconazole 500 ml ha^{-1}	30.70[cd] (33.64)[O]	49.23[e] (7.02)	19.07 (25.87)
Hexaconazole 500 ml ha^{-1} + Mancozeb 1kg ha^{-1}	24.47[b] (29.62)	43.41[c] (6.60)	6.50[a] (14.58)
Mancozeb 1kg ha^{-1}	27.27[bc] (31.46)	48.53[d] (6.97)	10.10[b] (18.40)
Hexaconazole 500 ml ha^{-1} + Monocrotophos 1000 ml ha^{-1}	8.03[a] (16.38)	11.33[a] (3.38)	11.70[b] (19.87)
Monocrotophos 1000 ml ha^{-1}	9.67[a] (18.01)	14.24[b] (3.79)	22.07[d] (27.99)
Control	35.53[d] (36.58)	63.67[f] (7.98)	47.93[e] (43.81)
CD	3.61	0.31	1.62

[*] Mean of three replications

[O] Values in the parentheses are arcsine transformed values

[#] Values in the parentheses are square root transformed values.

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 10. Compatibility of hexaconazole 5SC with insecticide for the management of pests and powdery mildew disease in chilli

Treatments	Thrips* (Mean population) in numbers	Aphid* (per cent leaf damage)	Powdery mildew* (Per cent Disease Index)
Hexaconazole 500 ml ha^{-1}	35.23^{d} (36.41) #	33.76^{e} (35.52)	18.73^{c} (25.62)
Hexaconazole 500 ml ha^{-1} + Mancozeb 1 kg ha^{-1}	26.87^{c} (31.22)	23.98^{c} (29.32)	7.60^{a} (15.96)
Mancozeb 1 kg ha^{-1}	28.80^{c} (32.45)	28.82^{d} (32.47)	11.20^{b} (19.53)
Hexaconazole 500 ml ha^{-1} + Monocrotophos 1000 ml ha^{-1}	8.73^{a} (17.10)	16.63^{a} (24.06)	15.50^{c} (23.16)
Monocrotophos 1000 ml ha^{-1}	15.70^{b} (23.3)	19.25^{b} (26.02)	28.50^{d} (32.26)
Control	42.13^{e} (40.47)	69.73^{f} (56.63)	52.53^{e} (46.45)
CD	3.34	1.07	2.96

* Mean of three replications

 # Values in the parentheses are arcsine transformed values

In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 11. Persistence of hexaconazole 5SC on okra fruits

Trial	Days after last spraying	Hexaconazole residues ($\mu g\ g^{-1}$)				
		500 ml ha^{-1}	750 ml ha^{-1}	1000 ml ha^{-1}	1500 ml ha^{-1}	Control
Trial 1	0	0.44	0.57	0.74	1.27	BDL
	1	0.32	0.41	0.51	0.73	BDL
	3	0.13	0.32	0.41	0.61	BDL
	5	0.10	0.21	0.25	0.33	BDL
	7	BDL	0.05	0.13	0.15	BDL
	10	BDL	BDL	BDL	0.04	BDL
Trial 2	0	0.43	0.50	0.55	0.64	BDL
	1	0.39	0.43	0.50	0.60	BDL
	3	0.26	0.32	0.38	0.47	BDL
	5	0.23	0.26	0.29	0.33	BDL
	7	0.19	0.23	0.24	0.26	BDL
	10	0.16	0.20	0.22	0.24	BDL
Trial 3	0	0.32	0.34	0.34	0.36	BDL
	1	0.25	0.29	0.31	0.33	BDL
	3	0.22	0.24	0.27	0.29	BDL
	5	0.19	0.22	0.24	0.27	BDL
	7	0.16	0.21	0.22	0.25	BDL
	10	0.16	0.18	0.20	0.23	BDL

BDL-Below Detectable Level

Determinability of the instrument -0.020 $\mu g\ g^{-1}$

Table 12. Dissipation of hexaconazole residues in okra

Trial	Days after last spraying	Dissipation (per cent)				Average dissipation (per cent)
		500 ml ha^{-1}	750 ml ha^{-1}	1000 ml ha^{-1}	1500 ml ha^{-1}	
Trial 1	0	-	-	-	-	-
	1	27.27	28.07	31.08	42.52	32.23
	3	70.45	43.86	44.59	51.97	52.72
	5	77.27	63.16	66.22	74.01	70.16
	7	95.45	91.22	82.43	88.19	89.32
	10	95.45	96.49	97.29	96.85	96.52
Trial 2	0	-	-	-	-	-
	1	09.30	14.00	09.09	06.25	09.66
	3	39.53	36.00	30.91	26.56	33.25
	5	46.51	48.00	47.27	48.44	47.55
	7	55.81	54.00	56.36	59.37	56.38
	10	62.79	60.00	60.00	62.50	61.32
Trial 3	0	-	-	-	-	-
	1	21.87	14.70	08.82	08.33	13.43
	3	31.25	29.41	20.59	19.44	25.17
	5	40.62	35.29	29.41	25.00	32.58
	7	50.00	38.23	35.29	30.55	38.52
	10	50.00	47.06	41.18	36.11	43.59

Table 16. Dissipation of hexaconazole residues in chilli

Trial	Days after last spraying	Dissipation (per cent)				Average dissipation (per cent)
		500 ml ha^{-1}	750 ml ha^{-1}	1000 ml ha^{-1}	1500 ml ha^{-1}	
Trial 1	0	-	-	-	-	-
	1	17.65	38.46	42.86	48.78	36.94
	3	41.18	42.31	54.28	58.54	49.08
	5	58.82	53.85	57.14	60.97	57.69
	7	82.35	84.61	82.86	70.73	80.14
	10	88.23	92.31	94.28	95.12	92.48
Trial 2	0	-	-	-	-	-
	1	60.00	50.00	16.66	19.35	36.50
	3	60.00	83.33	58.33	32.26	58.48
	5	60.00	83.33	83.33	77.42	76.02
	7	60.00	83.33	91.66	80.64	78.91
	10	60.00	83.33	91.66	93.55	82.13
Trial 3	0	-	-	-	-	-
	1	33.33	23.08	35.00	16.66	27.02
	3	33.33	53.85	60.00	55.55	50.68
	5	33.33	84.61	90.00	94.44	75.59
	7	33.33	84.61	90.00	94.44	75.59
	10	33.33	84.61	90.00	94.44	75.59

Table 15. Persistence of hexaconazole 5SC on chilli fruits

Trial	Days after last spraying	Hexaconazole residues (μg g^{-1})				
		500 ml ha^{-1}	750 ml ha^{-1}	1000 ml ha^{-1}	1500 ml ha^{-1}	Control
Trial 1	0	0.17	0.26	0.35	0.41	BDL
	1	0.14	0.16	0.20	0.21	BDL
	3	0.10	0.15	0.16	0.17	BDL
	5	0.07	0.12	0.15	0.16	BDL
	7	0.03	0.04	0.06	0.12	BDL
	10	BDL	BDL	BDL	BDL	BDL
Trial 2	0	0.05	0.12	0.24	0.31	BDL
	1	BDL	0.06	0.20	0.25	BDL
	3	BDL	BDL	0.10	0.21	BDL
	5	BDL	BDL	0.04	0.07	BDL
	7	BDL	BDL	BDL	0.06	BDL
	10	BDL	BDL	BDL	BDL	BDL
Trial 3	0	0.03	0.13	0.20	0.36	BDL
	1	BDL	0.10	0.13	0.30	BDL
	3	BDL	0.06	0.08	0.16	BDL
	5	BDL	BDL	BDL	BDL	BDL
	7	BDL	BDL	BDL	BDL	BDL
	10	BDL	BDL	BDL	BDL	BDL

BDL-Below Detectable Level

Determinability of the instrument - 0.020 μg g^{-1}

Table 21. Bioefficacy of hexaconazole 5 SC against okra powdery mildew under glass house conditions

Treatment	Per cent disease index*			Per cent reduction over control		
	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III
Hexaconazole 500 ml ha^{-1}	36.10[d] (36.93)	40.23[d] (39.37)	36.20[d] (36.98)	58.63	55.61	60.78
Hexaconazole 750 ml ha^{-1}	31.07[c] (33.87)	34.37[c] (35.89)	31.93[c] (34.41)	64.40	62.08	65.41
Hexaconazole 1000 ml ha^{-1}	26.07[b] (30.70)	29.47[b] (32.88)	26.52[b] (30.99)	70.13	67.48	71.27
Hexaconazole 1500 ml ha^{-1}	19.73[a] (26.37)	22.57[a] (28.36)	20.40[a] (26.85)	77.39	75.10	77.89
Mancozeb 1Kg ha^{-1}	38.63[e] (38.43)	44.17[c] (41.65)	38.45[e] (38.32)	55.73	51.26	58.34
Propiconazole 1000 ml ha^{-1}	41.13[f] (39.89)	47.10[f] (43.34)	42.84[f] (40.85)	52.87	48.03	53.58
Dinocap 1000 ml ha^{-1}	43.40[g] (41.21)	49.12[g] (44.48)	45.38[g] (42.35)	50.27	45.80	50.83
Wettable sulphur 1500 ml ha^{-1}	45.23[h] (42.26)	51.07[h] (45.61)	47.22[g] (43.41)	48.17	43.65	48.84
Control (sterile water)	87.27[I] (69.11)	90.63[i] (72.21)	92.30[h] (73.94)	-	-	-
CD	1.14	1.06	1.15			

* Mean of three replications
Values in the parentheses are arcsine transformed values
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 22. Bioefficacy of hexaconazole 5 SC against chilli powdery mildew under glass house conditions

Treatment	Per cent disease index*			Per cent reduction over control		
	Trial I	Trial II	Trial III	Trial I	Trial II	Trial III
Hexaconazole 500 ml ha^{-1}	38.27^{d} (38.21)	34.67^{d} (36.07)	35.45^{d} (36.54)	57.63	62.85	62.53
Hexaconazole 750 ml ha^{-1}	34.33^{c} (35.87)	31.43^{c} (34.10)	32.24^{c} (34.59)	61.99	66.32	65.91
Hexaconazole 1000 ml ha^{-1}	27.50^{b} (31.63)	25.53^{b} (30.35)	25.80^{d} (30.53)	69.56	72.64	72.73
Hexaconazole 1500 ml ha^{-1}	20.60^{a} (26.99)	16.70^{a} (24.12)	18.58^{a} (25.53)	77.19	82.11	80.36
Mancozeb 1Kg ha^{-1}	40.27^{e} (39.40)	36.53^{d} (37.19)	37.36^{e} (37.68)	55.42	60.86	60.51
Propiconazole 1000 ml ha^{-1}	42.23^{f} (40.53)	38.80^{e} (38.53)	39.17^{e} (38.74)	53.25	58.43	58.59
Tridemorph 1000 ml ha^{-1}	45.20^{g} (42.24)	42.23^{f} (40.53)	42.58^{f} (40.73)	49.96	54.75	54.98
Wettable sulphur 1500 ml ha^{-1}	47.13^{h} (43.36)	44.70^{g} (41.96)	43.18^{f} (41.08)	47.82	52.10	54.38
Control (sterile water)	90.33^{I} (71.92)	93.33^{h} (75.10)	94.60^{g} (76.63)	-	-	-
CD	1.05	1.17	1.11			

* Mean of three replications
Values in the parentheses are arcsine transformed values
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 23. ***In vitro*** **study of spore germination of** ***Erysiphe cichoracearum*** **DC in different concentrations of hexaconazole 5 SC**

Treatment	Per cent spore germination*		
	2 days after treatment	3 days after treatment	15 days after treatment
Hexaconazole 100 ppm	51.77[d] (7.20)	44.17[d] (6.65)	20.27[d] (4.51)
Hexaconazole 500 ppm	40.80[c] (6.39)	35.93[c] (6.00)	12.20[c] (3.50)
Hexaconazole 1000 ppm	26.87[b] (5.19)	21.57[b] (4.65)	7.10[b] (2.68)
Hexaconazole 2000 ppm	14.93[a] (3.88)	8.23[a] (2.89)	3.03[a] (1.77)
Mancozeb 1000 ppm	56.73[e] (7.54)	50.33[e] (7.10)	23.30[e] (4.84)
Control (Sterile water)	78.66[f] (8.87)	88.33[f] (9.40)	96.33[f] (9.82)

CD for t x d means = 1.42

* Mean of three replications
Values in the parentheses are square root transformed values
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 24. ***In vitro*** **study of spore germination of** ***Leveillula taurica*** **in different concentrations of hexaconazole 5SC**

Treatment	Per cent spore germination*		
	2 days after treatment	3 days after treatment	15 days after treatment
Hexaconazole 100 ppm	31.90[d] (5.66)	23.47[d] (4.85)	12.00[d] (3.48)
Hexaconazole 500 ppm	25.70[c] (5.08)	16.33[c] (4.05)	9.00[c] (3.02)
Hexaconazole 1000 ppm	19.53[b] (4.43)	11.23[b] (3.37)	5.00[b] (2.26)
Hexaconazole 2000 ppm	15.43[a] (3.94)	6.10[a] (2.49)	2.00[a] (1.45)
Mancozeb 1000 ppm	33.97[e] (5.83)	25.50[e] (5.06)	17.00[e] (4.13)
Control (Sterile water)	71.00[f] (8.43)	81.67[f] (9.04)	86.00[f] (9.28)

CD for t x d means = 1.30

* Mean of three replications
Values in the parentheses are square root transformed values

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 27. Effect of fungicides on the contents of total phenol and soluble protein in okra leaves

Treatment	Total phenol content* (mg/100g)		Soluble protein content* (mg/100g)	
	24 h after treatment	72 h after treatment	24 h after treatment	72 h after treatment
Hexaconazole 500 ml ha^{-1}	343.33[de]	436.67[d]	550.00[cd]	556.67[bcd]
Hexaconazole 750 ml ha^{-1}	386.60[bc]	510.00[c]	560.00[bc]	568.33[bc]
Hexaconazole 1000 ml ha^{-1}	406.67[b]	613.30[b]	571.67[b]	583.31[b]
Hexaconazole 1500 ml ha^{-1}	540.00[a]	740.00[a]	604.00[a]	613.40[a]
Mancozeb 1Kg ha^{-1}	366.69[cd]	400.00[e]	540.00[d]	546.70[cd]
Propiconazole 1000 ml ha^{-1}	340.00[de]	386.68[e]	520.00[e]	530.00[de]
Dinocap 1000 ml ha^{-1}	330.00[ef]	376.60[e]	510.00[ef]	513.40[e]
Wettable sulphur 1500 ml ha^{-1}	310.00[f]	346.65[f]	500.00[f]	506.72[e]
Control (Sterile water)	273.32[g]	310.00[g]	480.00[g]	433.28[f]
CD for t x d means	27.82		21.44	

* Mean of three replications
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 28. Effect of fungicides on the contents of total phenol and soluble protein in chilli leaves

Treatments	Total phenol content* (mg/100g)		Soluble protein content* (mg/100g)	
	24 h after treatment	72 h after treatment	24 h after treatment	72 h after treatment
Hexaconazole 500 ml ha^{-1}	520.00[e]	620.00[d]	433.30[c]	461.72[cd]
Hexaconazole 750 ml ha^{-1}	560.00[d]	671.70[c]	443.50[c]	491.68[c]
Hexaconazole 1000 ml ha^{-1}	746.68[b]	763.32[b]	506.70[b]	555.00[b]
Hexaconazole 1500 ml ha^{-1}	813.30[a]	850.00[a]	545.00[a]	628.30[a]
Mancozeb 1Kg ha^{-1}	646.69[c]	656.73[c]	435.00[c]	450.00[de]
Propiconazole 1000 ml ha^{-1}	620.00[c]	623.00[d]	423.20[c]	440.00[de]
Tridemorph 1000 ml ha^{-1}	520.00[e]	550.00[e]	413.30[cd]	425.00[ef]
Wettable sulphur 1500 ml ha^{-1}	493.31[e]	518.29[f]	393.28[de]	405.00[f]
Control (Sterile water)	410.00[f]	383.41[g]	374.20[e]	370.00[g]
CD for t x d means	25.70		29.35	

* Mean of three replications
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 39. Residues of hexaconazole in the harvested okra fruits and in soil

Treatment	Hexaconazole residues (µg g^{-1})					
	Trial I		Trial II		Trial III	
	Fruits	Soil	Fruits	Soil	Fruits	Soil
Hexaconazole 500 ml ha^{-1}	BDL	BDL	BDL	BDL	BDL	BDL
Hexaconazole 750 ml ha^{-1}	BDL	BDL	BDL	BDL	**BDL**	BDL
Hexaconazole 1000 ml ha^{-1}	BDL	BDL	BDL	BDL	BDL	BDL
Hexaconazole 1500 ml ha^{-1}	BDL	BDL	BDL	BDL	BDL	BDL
Control	BDL	BDL	BDL	BDL	BDL	BDL

BDL – Below Detectable Level

Determinability of the instrument for okra fruits (25 g) = 0.020 µg g^{-1}

Determinability of the instrument for soil (50 g) = 0.01 µg g^{-1}

Table 40. Residues of hexaconazole in the harvested chilli fruits and in soil

Treatment	Hexaconazole residues (µg g^{-1})					
	Trial I		Trial II		Trial III	
	Fruits	Soil	Fruits	Soil	Fruits	Soil
Hexaconazole 500 ml ha^{-1}	BDL	BDL	BDL	BDL	BDL	BDL
Hexaconazole 750 ml ha^{-1}	BDL	BDL	BDL	BDL	**BDL**	BDL
Hexaconazole 1000 ml ha^{-1}	BDL	BDL	BDL	BDL	BDL	BDL
Hexaconazole 1500 ml ha^{-1}	BDL	BDL	BDL	BDL	BDL	BDL
Control	BDL	BDL	BDL	BDL	BDL	BDL

BDL – Below Detectable Level

Determinability of the instrument for okra fruits (25 g) = 0.020 µg g^{-1}

Determinability of the instrument for soil (50 g) = 0.01 µg g^{-1}

Table 41. Recovery percentage of hexaconazole in fortified okra and chilli fruits

Crop	Recovery per cent of Hexaconazole			Mean recovery (per cent)	Recovery factor (RF)
	Fortification level (ppm)				
	0.5	3.0	5.0		
Okra	81.80	83.30	85.00	83.40	1.20
Chilli	82.00	83.00	85.54	83.51	1.19
Soil	80.00	83.00	85.60	82.86	1.20

Determinability of the instrument for fruits (25 gm) = 0.020 μg g^{-1}

Determinability of the instrument for soil (50 gm) = 0.01 μg g

Table 37. Effect of different concentration of hexaconazole on biocontrol agents

Hexazonazole concentrations (ppm)	*Diameter of mycelial growth (cm)**			
	Trichoderma viride		**Trichoderma harzianum**	
	Days after treatment			
	3	5	3	5
0.001	6.84[a]	8.97[a]	7.26[b]	8.91[a]
0.01	8.77[a]	8.84[b]	7.20[b]	8.80[a]
0.05	6.60[ab]	8.60[bc]	6.83[c]	8.60[b]
0.10	6.37[b]	8.43[c]	6.66[c]	8.23[c]
0.50	5.63[c]	6.66[d]	6.00[d]	7.50[d]
1.0	2.97[d]	3.10[e]	3.33[e]	3.60[e]
Control	6.73[a]	8.96[a]	7.66[a]	8.93[a]
CD for t x d means	0.27		0.20	

* Mean of three replications
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 38. Effect of different concentration of hexaconazole on other pathogens

Hexazonazole concentrations (ppm)	***Diameter of mycelial growth (cm)****			
	Alternaria capsici		**Colletotrichum capsici**	
	Days after treatment			
	3	5	3	5
0.001	6.16^{de}	8.36^{f}	5.86^{f}	7.80^{f}
0.01	6.00^{d}	6.90^{e}	5.53^{e}	7.13^{a}
0.05	5.73^{c}	6.20^{d}	5.13^{d}	6.33^{d}
0.10	5.46^{bc}	5.63^{c}	4.83^{c}	5.53^{c}
0.50	4.93^{b}	4.96^{b}	3.63^{b}	4.36^{b}
1.0	2.56^{a}	2.63^{a}	2.33^{a}	2.43^{a}
Control	6.36^{e}	8.83^{g}	6.10^{f}	8.50^{g}
CD for t x d means	0.22		0.28	

* Mean of three replications
In a column, means followed by a common letter are not significantly different at 5% level by DMRT

RESEARCH FINDINGS

Bioefficacy and Persistence of Hexaconazole 5 SC against Okra Powdery mildew (*Erysiphe cichoracearum* DC) and Chilli Powdery mildew (*Leveillula taurica* (Lev.) Arn.)

Student : Mareeswari, P. **Chairman : Dr. R. Samiyappan**

Hexaconazole 5 SC at 500 ml, 750 ml, 1000 ml and 1500 ml ha^{-1} was highly effective against the powdery mildew disease in okra and chilli both in green-house and field experiments. Besides, the yield was also increased due to the test chemical spray. The test chemical was highly compatible with monocrotophos and mancozeb and its synergistic action was effective against the pests and powdery mildew disease. The persistence of the test chemical was observed up to ten days after third spray in okra fruits and it was up to seven days in chilli fruits at high concentration of 1500 ml ha^{-1}. No phytotoxic effect was observed in all the doses of hexaconazole 5 SC. The test chemical at 2000 ppm showed the least per cent spore germination of *E. cichoracearum* and *L. taurica*. The chlorophyll content, total phenol and soluble protein content were increased in okra and chilli leaves when these crops were sprayed with the test chemical. The increased activity of peroxidase, polyphenol oxidase and phenylalanine ammonialyase was observed and the induction of peroxidase and polyphenol oxidase enzyme was also observed immediately after the hexaconazole spray. Spraying of hexaconazole resulted in increase in the fungal and bacterial population in the phylloplane and rhizosphere regions. The compatibility studies of hexaconazole with biocontrol agents indicate that it was highly inhibitory to *Trichoderma viride* and *T. harzianum* even at the concentration of 1.0 ppm. However the growth of *Pseudomonas fluorescens* and *Bacillus subtilis* was unaffected. Hexaconazole at 0.5 and 1.0 ppm was

highly inhibitory to *Alternaria capsici* and *Colletotrichum capsici*. There were no residues of hexaconazole detected in the fruits of okra, chilli and also in soil.

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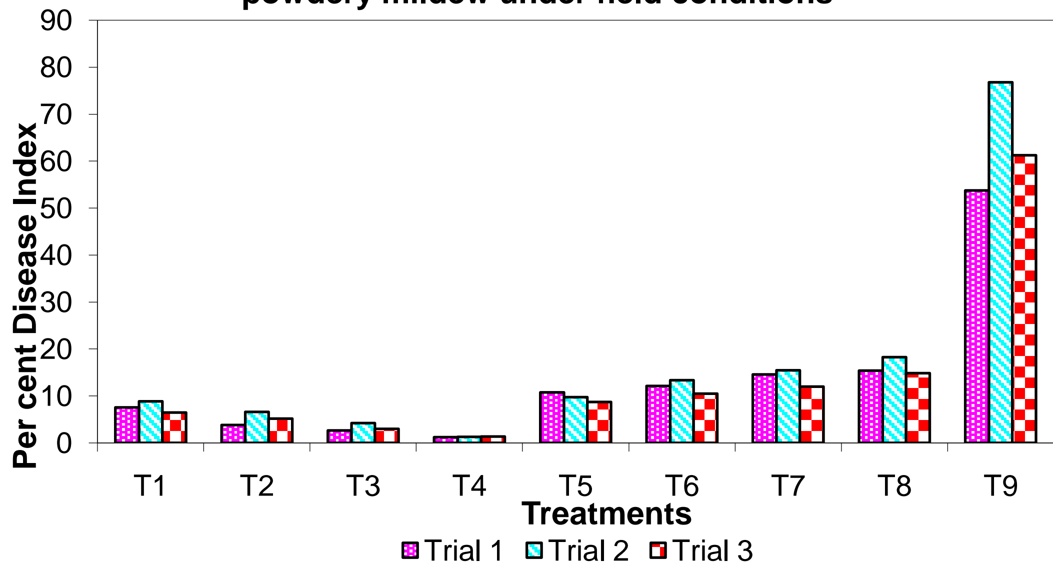
* Originals not seen

Fig. 2. Bioefficacy of hexaconazole 5SC against okra powdery mildew under field conditions



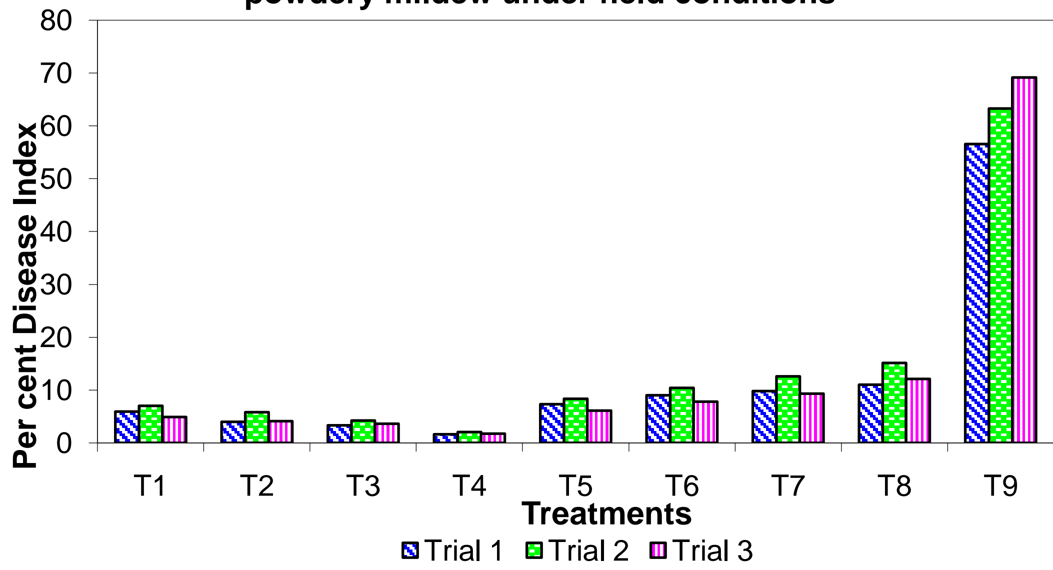
T1 - Hexaconazole 500 ml ha^{-1}

T4 - Hexaconazole 1500 ml ha^{-1}

T7 - Dinocap 1000 ml ha^{-1}

T8 - Wettable sulphur

Fig. 3. Bioefficacy of hexaconazole 5SC against chilli powdery mildew under field conditions



T1 - Hexaconazole 500 ml ha^{-1} T4 - Hexaconazole 1500 ml ha^{-1} T7 - Tridemorph 1000 ml ha^{-1}

Fig. 4. Compatibility of hexaconazole 5SC with insecticide against the pests and powdery mildew disease in okra

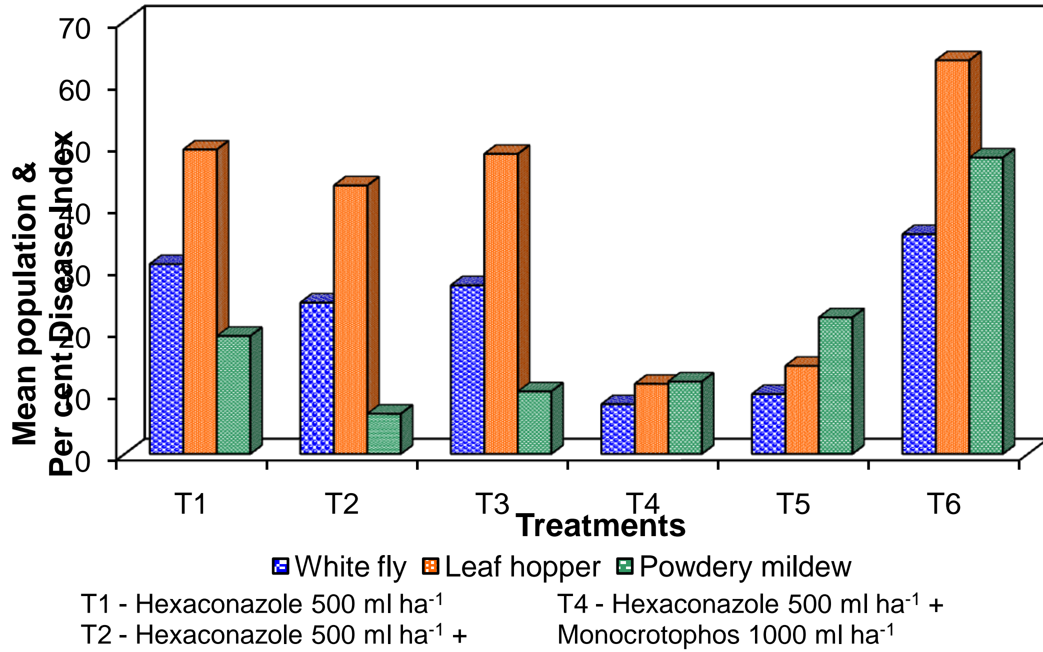


Fig. 5. Compatibility of hexaconazole 5SC with insecticide against the pests and powdery mildew disease in chilli

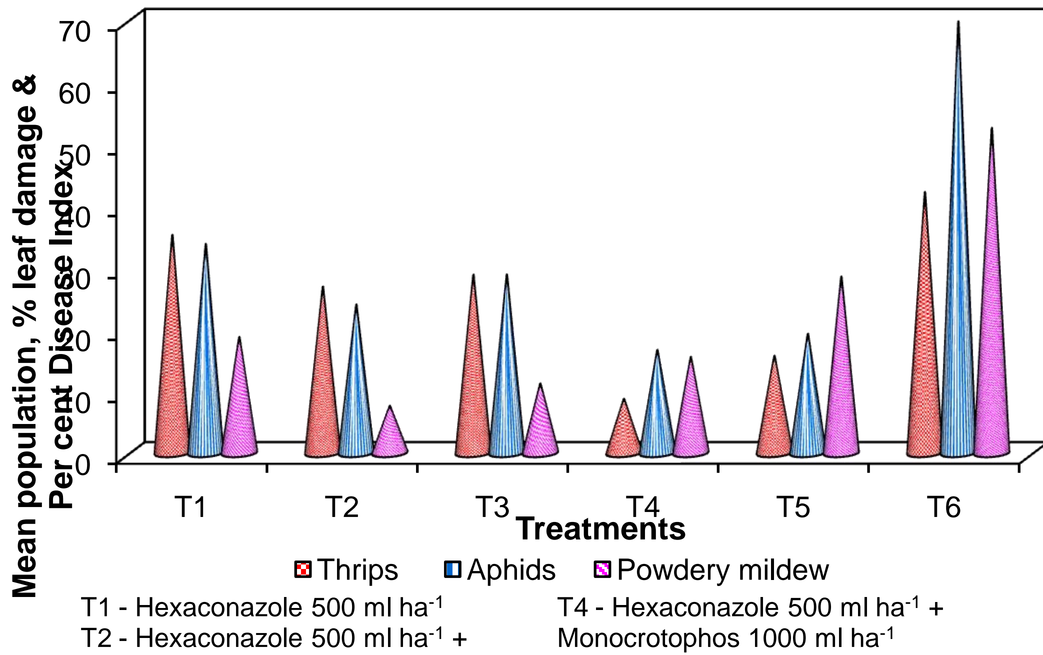


Fig. 6. Persistence and dissipation of hexaconazole 5SC on okra fruits

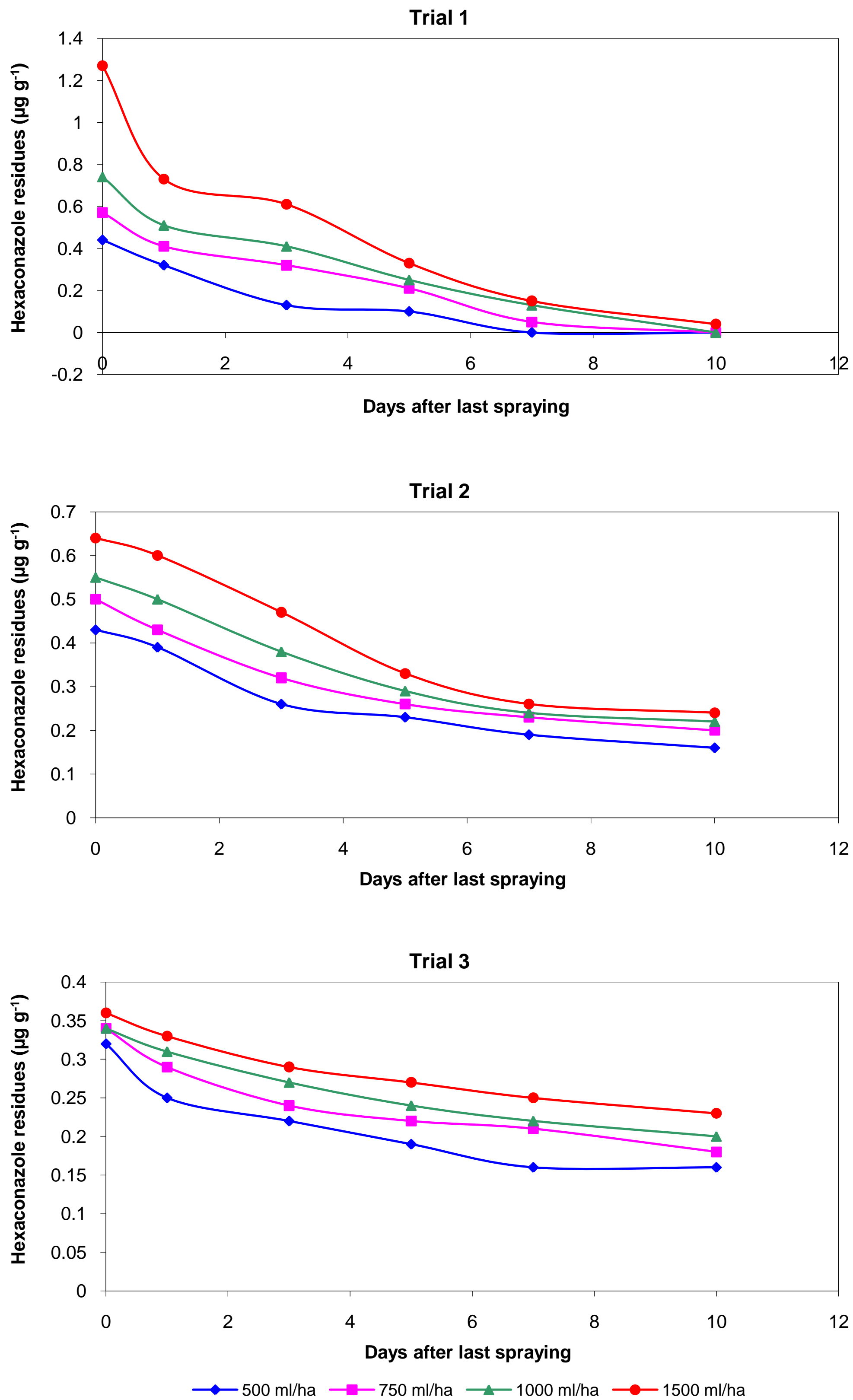
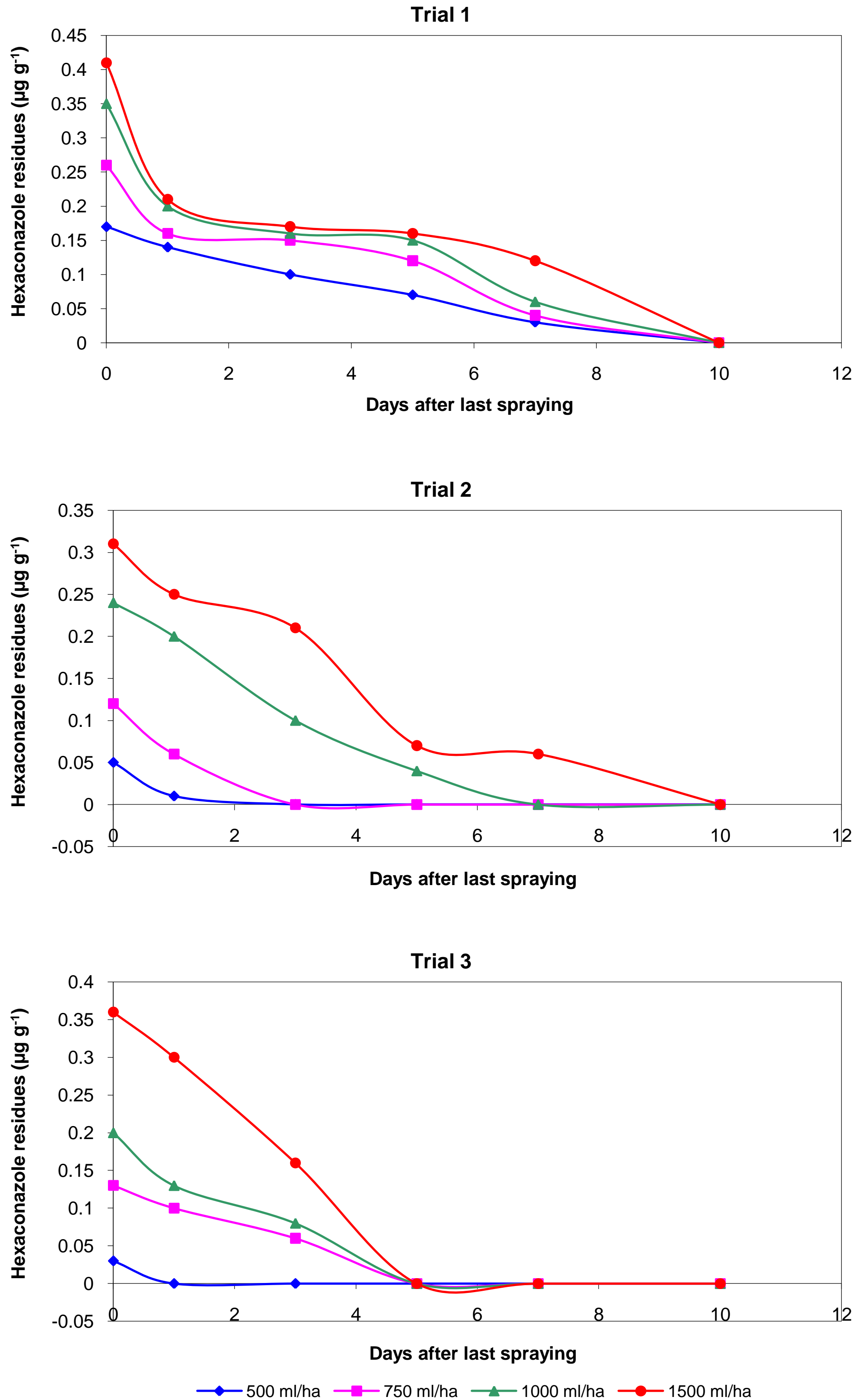


Fig. 7. Persistence and dissipation of hexaconazole 5SC on chilli fruits



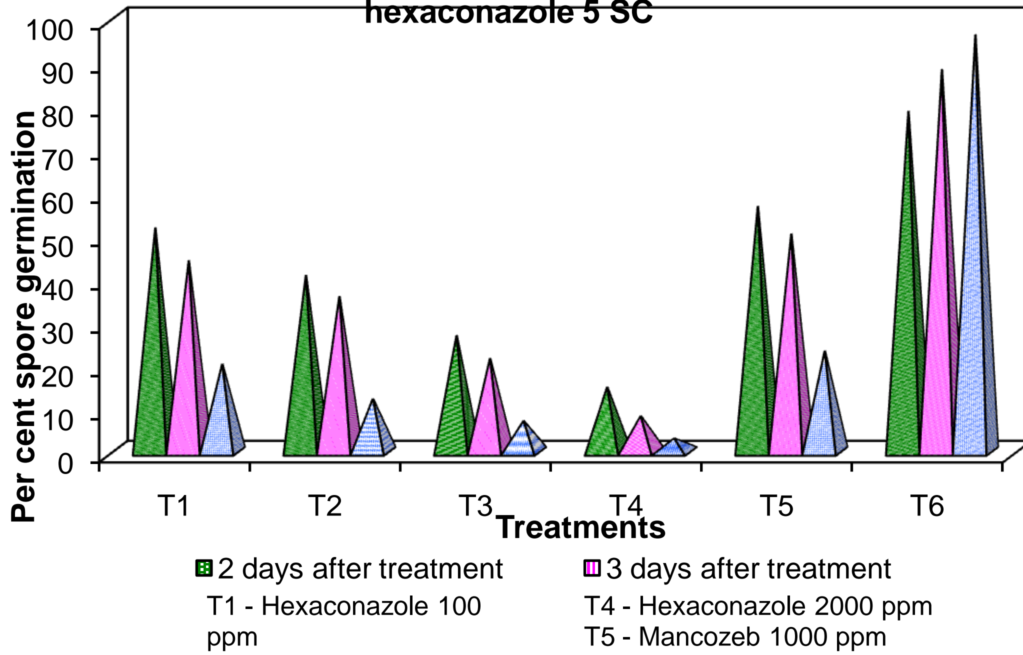


Fig. 8. Per cent spore germination of *Erysiphe cichoracearum* DC in different concentrations of hexaconazole 5 SC

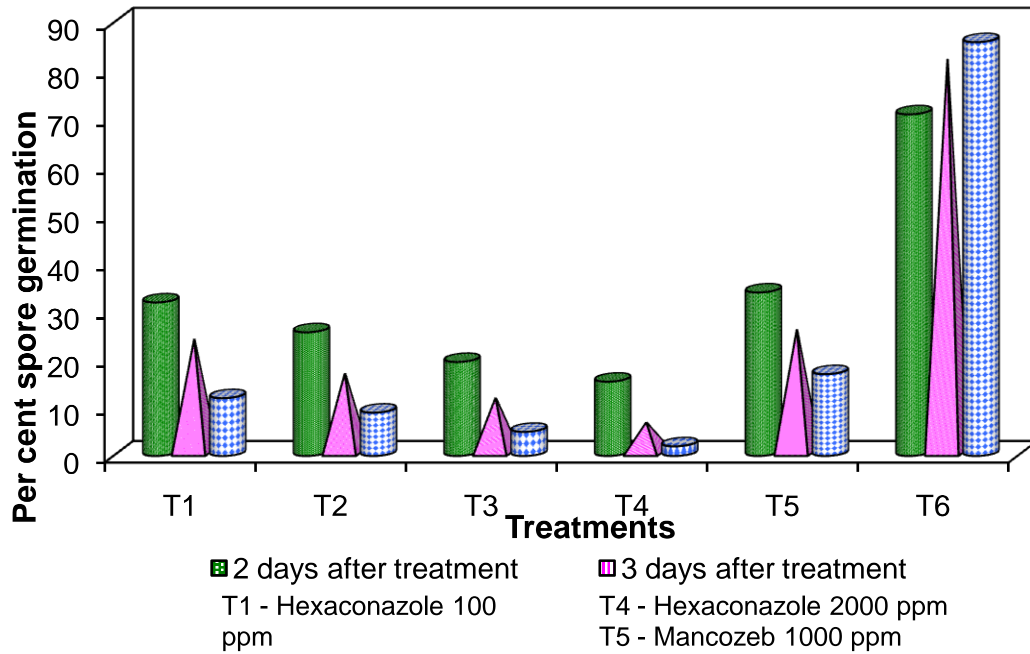


Fig. 9. Per cent spore germination of *Leveillula taurica* in different concentrations of hexaconazole 5 SC
Per cent spore germination
0
10
20
30
40
50
60
70
80
90
T1
T2
T3
T4
T5
T6
Treatments
2 days after treatment
3 days after treatment
T1 - Hexaconazole 100 ppm
T4 - Hexaconazole 2000 ppm
T5 - Mancozeb 1000 ppm

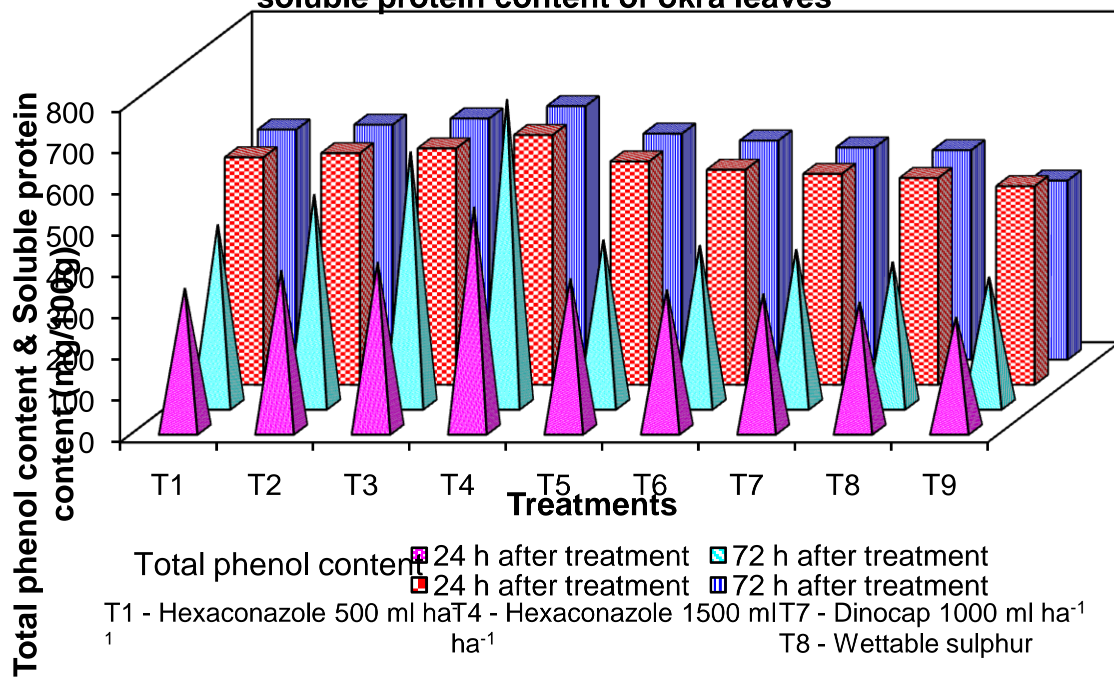
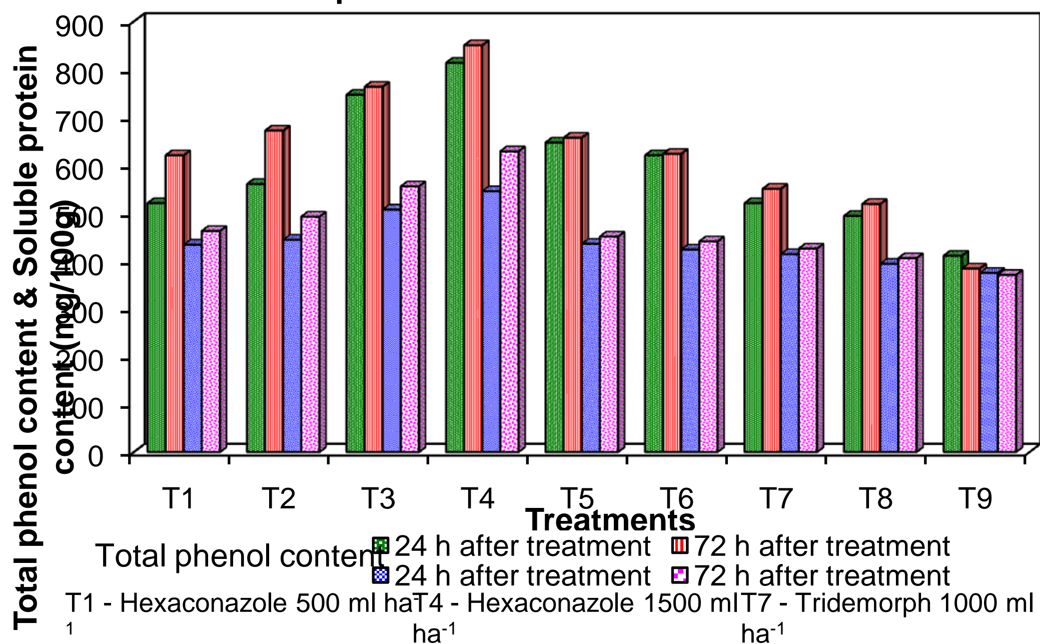


Fig. 10. Effect of hexaconazole 5 SC on total phenol and soluble protein content of okra leaves
Total phenol content & Soluble protein content (mg/100g)
0
100
200
300
400
500
600
700
800
T1
T2
T3
T4
T5
T6
T7
T8
T9
Treatments
Total phenol content
24 h after treatment
72 h after treatment
24 h after treatment
72 h after treatment
T1 - Hexaconazole 500 ml ha-1
T4 - Hexaconazole 1500 ml ha-1
T7 - Dinocap 1000 ml ha-1
T8 - Wettable sulphur

Fig. 11. Effect of hexaconazole 5 SC on total phenol and soluble protein content of chilli leaves



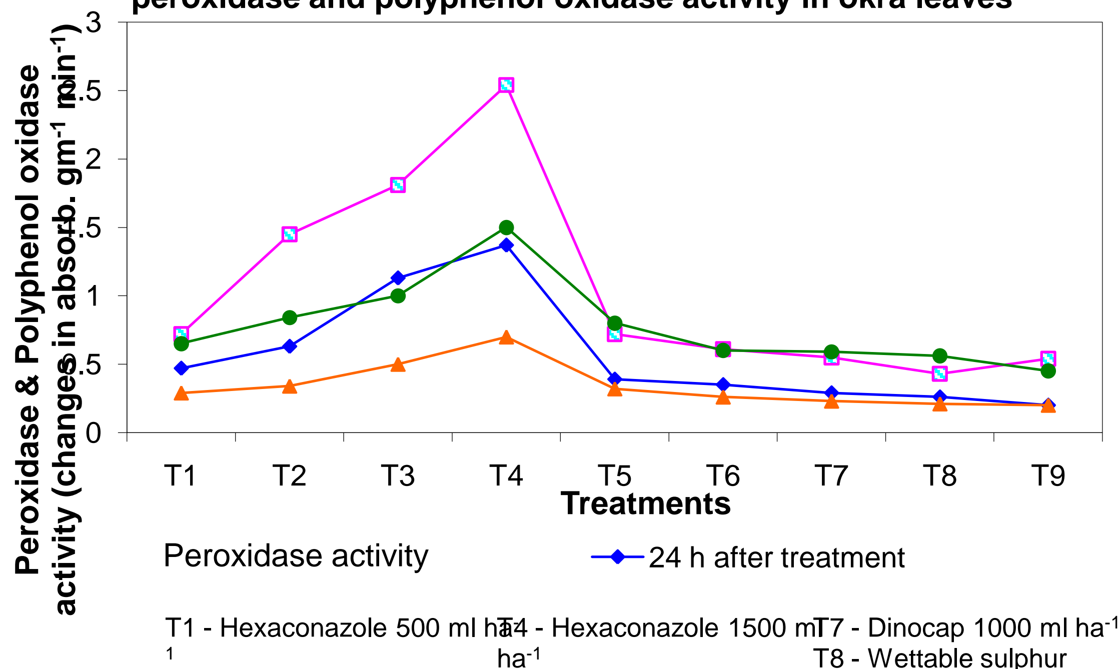


Fig. 12. Effect of hexaconazole 5 SC on induction of peroxidase and polyphenol oxidase activity in okra leaves
Peroxidase & Polyphenol oxidase activity (changes in absorb. gm-1 min-1)
3
2.5
2
1.5
1
0.5
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
Treatments
Peroxidase activity
24 h after treatment
T1 - Hexaconazole 500 ml ha-1
T4 - Hexaconazole 1500 ml ha-1
T7 - Dinocap 1000 ml ha-1
T8 - Wettable sulphur

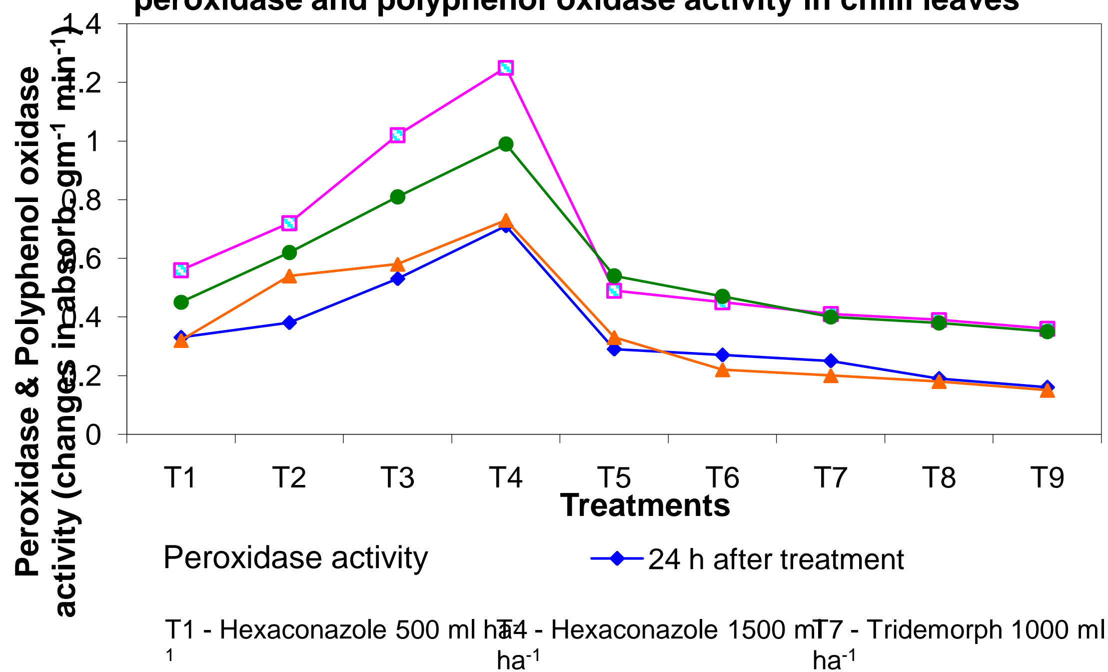


Fig. 13. Effect of hexaconazole 5 SC on induction of peroxidase and polyphenol oxidase activity in chilli leaves
Peroxidase & Polyphenol oxidase activity (changes in absorb. gm-1 min-1)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
T1
T2
T3
T4
T5
T6
T7
T8
T9
Treatments
Peroxidase activity
24 h after treatment
T1 - Hexaconazole 500 ml ha-1
T4 - Hexaconazole 1500 ml ha-1
T7 - Tridemorph 1000 ml ha-1