TOLERANCE OF ENTOMOPATHOGENIC FUNGI *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno AND *Lecanicillium lecanii* (Zimm.) Zare and Gams TO ABIOTIC STRESS

by

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(2017-21-036)

THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"TOLERANCE OF ENTOMOPATHOGENIC FUNGI *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno AND *Lecanicillium lecanii* (Zimm.) Zare and Gams TO ABIOTIC STRESS"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Tejaswi G Gowda

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	:	Per cent
@	:	at the rate of
µL	:	microlitre
µm	:	micro metre
CD	:	Critical difference
cm	:	Centimetre
DAI	:	Days after inoculation
DAT	:	Days after treatment
et al.	:	Co-workers
g	:	Gram
h	:	Hour
KAU	:	Kerala Agricultural University
mg	:	Milligram
min	:	Minutes
mL	:	millilitre
mm	:	Millimetre
mm day^{-1}	:	Millimetre per day
°C	:	Degree Celsius
PCR	:	Polymerase chain reaction
PDA	:	Potato dextrose agar
PDB	:	Potato dextrose broth
rpm	:	Revolutions per minute
Sec	:	Second
Sp. or spp	:	Species (Singular and plural)

S.E	:	Standard error
SC	:	Soluble concentrate
S.EM	:	Standard error of mean
UV	:	Ultraviolet
viz.	:	Namely

Introduction

1. INTRODUCTION

Over the past 50 years, crop protection has relied heavily on synthetic chemical pesticides worldwide. The growing concern regarding their ill effects in the ecosystem and the emerging pesticide resistance issues led to a remarkable shift to the use of microbial pesticides. Of these, entomopathogenic fungi are the only group that can cause disease in sucking pests, owing to their unique mode of entry. The most commonly used entomopathogenic fungi are *Beauveria bassiana* (Bals.) Vuill., *Lecanicillium lecanii* (Zimm.) Zare and Gams, *Metarhizium anisopliae* (Metschn.) Sorokin, *Metarhizium acridum* (Driver and Milner) *Isaria fumosorosea* Wize and *Hirsutella thompsonii* (Fisher).

In spite of their merits, biopesticides comprise only a small share of the total crop protection market globally. The value of share is about $3 billion worldwide, which accounts for five per cent of the total pesticide market (Kumar *et al.*, 2021). Among the major factors responsible for the lower share of biopesticides in the global pesticide market, abiotic stresses that limit their efficacy under field conditions occupies a major role.

Virulence of entomopathogenic fungi to infect and colonise host insects is mainly determined by the abiotic factors. This fact limits the 'window of opportunity' of entomopathogenic fungi to infect an insect host, thus reducing their efficacy (Yeo *et al.*, 2003). Among the various abiotic stresses, temperature and Ultra-Violet (UV) rays are the most detrimental for survivability of fungi. Usually, entomopathogenic fungi are mesophiles, *i.e.*, their optimum temperature range for better growth, development, pathogenicity and survivability is 25 °C to 30°C (Maniania *et al.*, 2008). Disease development rate and extent of infection are higher under field conditions at the optimum temperature than at lower or higher temperatures.

Ultraviolet A and Ultraviolet B radiations from sunlight are the most detrimental factors which affect the viability of entomopathogenic fungi when

applied on solar-exposed sites such as the phylloplane, for pest control. While UVA causes only indirect damage to DNA through catalysing the formation of chemical intermediates such as reactive oxygen species (ROS), UVB acts directly in DNA inducing the formation of several photoproducts (Braga *et.al.*, 2001a).

Along with natural abiotic stress, entomopathogenic fungi are also affected adversely by the exposure to pesticides such as insecticides, fungicides and herbicides which either impair the pathogenicity by affecting spore germination or affect their epizootic potential by inhibiting their growth and development.

The genus *Lecanicillium* is well known for its efficacy in managing homopteran sucking pests. *L. lecanii* is the most popularly used species for the management of soft bodied, colonising sucking insect pests *viz.,* aphids, scales and mealy bugs. However, they are reported to be slow in action and sensitive to environment. *L. saksenae* is an indigenous isolate from soils of Vellayani, Thiruvananthapuram, which has wider host range and adaptability to environmental conditions of Kerala (Rani *et al.,* 2014; 2015). Pathogenicity of *L. saksenae* was established by detecting the cuticle degrading enzymes (Jasmy, 2016) and field efficacy was ascertained by Sankar and Rani (2018). Several primary and secondary metabolites contributing to its pathogenicity and virulence were profiled by Sreeja and Rani (2019). It was found to be capable of endophytic colonization in rice and cowpea (Divyashree, 2019).

A comparative analysis on tolerance of these two species *L. saksenae* and *L. lecanii*, to abiotic stresses would pave way to the right selection of species for location specific management of sucking pests. Information gathered on these aspects would enable development of improved strains through artificial selection. Developing strains tolerant to pesticides, temperature and UV exposure enables better performance of these bioagents under open field conditions. Therefore, the present investigation entitled "Tolerance of entomopathogenic fungi *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and

Lecanicillium lecanii (Zimm.) Zare and Gams to abiotic stress" was carried out with the following objectives.

- Screening for tolerance of *L. saksenae* and *L. lecanii* to insecticides and fungicides
- Induction of insecticide and fungicide tolerance
- Screening for temperature and UV tolerance
- Induction of temperature tolerance and UV tolerance
- Molecular analysis of improved strains
- Pathogenicity assay of improved strains

Review of Literature

2. REVIEW OF LITERATURE

Fungal entomopathogens are important microbial insecticides that are used to control a wide range of insect pests. Entomopathogenic fungi, unlike other entomopathogens, infect insects through direct contact with the host cuticle. *Beauveria bassiana* (Bals.) Vuill., *Lecanicillium lecanii* (Zimm.) Zare and Gams, *Metarhizium anisopliae* (Metschn.) Sorokin, *Isaria fumosorosea* Wize, *Nomuraea rileyi* (Farlow) are the common entomopathogenic fungi that have been extensively utilised for the management of a range of insect pests.

Genus *Lecanicillium* is widely being used for managing the sucking pests of several crops. In green house *L. lecanii* is an efficient biocontrol agent for the management of *Trialeurodes vaporariorum* Westwood (Kim *et al.,* 2002). It is the most widely distributed species, capable of causing epizootics in tropical and subtropical regions where warm humid conditions are prevalent (Nunez *et al.,* 2008).

Lecanicillium saksenae (Kushwaha) *Kurihara and Sukarno* (ITCC Accession No. LsVs1 7714), an indigenous entomopathogenic fungus isolated from the soils of Vellayani, Kerala, (Rani *et al.,* 2014; 2015), was found to be more adaptive to the local environmental conditions and amenable to mass production. Its pathogenicity could be established by detecting the cuticle degrading enzymes and toxins (Jasmy, 2016). Aside from infecting homopterans its spectrum of action is widened to heteropterans too, with high virulence and speed of kill unlike *L. lecanii*. Sankar (2017) computed its LD_{50} value as 10^7 spores mL^{-1} and LT_{50} 19.5 h. It was efficient in managing *Leptocorisa acuta* (Thunberg) in rice field causing 75.04 per cent reduction in population (Sankar and Rani, 2018). It was found to be compatible with new generation insecticides such as flubendiamide 39.5% SC, chlorantraniliprole 18.5% SC, thiamethoxam 25% WG and imidacloprid 17.5% SL (Keerthana, 2019). *L. saksenae* is a versatile fungus, even capable for its endophytic activity in rice and cowpea

(Divyashree, 2019). Investigations carried out to profile its metabolites revealed that it produces primary metabolites such as protease, chitinase, lipase and chitosinase. It is also a treasure of many secondary metabolites which includes 22 insecticidal compounds, seven nematicidal compounds and 20 antimicrobial compounds (Sreeja and Rani, 2019; Sreeja, 2020).

The virulence and viability of entomopathogens are mainly deterred by biotic and abiotic stress factors. Abiotic factors could impose great challenge to their field application. Upon field application, they are exposed to an array of abiotic stress like UV irradiance, temperature, rainfall, relative humidity and pesticides (Rangel *et al.,* 2005, 2006). The extremities in any of the above results in failure of microbial pest control. The abiotic factors that would impair the field performance of entomopathogenic fungi and the research works related to improvement of their tolerance to these stress factors, is reviewed in this chapter.

2.1 TOLERANCE TO PESTICIDES

2.1.1 Insecticides

Tolerance of the *L. saksenae* and *L. lecanii* to insecticides tested in this study is reviewed here under. The effect of inhibitory factors on fungi is usually assessed based on its growth parameters such as radial growth of mycelia, sporulation and conidial germination.

2.1.1.1 New Generation Insecticides

2.1.1.1.1 Radial Growth

Medium poisoned with imidacloprid 200 SL 0.0045 % resulted in 0.56 to 1.28 per cent growth inhibition in *B. bassiana* (Rajanikanth *et al.,* 2010). Imidacloprid 17.8% SL 0.003 to 0.006 % resulted in 1.16 to 3.37 per cent inhibition in *Lecanicillium longisporum* (Petch) Zare and Gams (Panahi *et al.,* 2012).

Thiamethoxam 250 WG 0.16% was compatible with *L. lecanii* in terms of radial growth (Filho *et al.,* 2001). Growth reduction of 21 per cent in *B. bassiana* and five per cent in *M. anisopliae* was observed in medium poisoned with thiamethoxam 25% WG 0.005% (Neves *et al.,* 2001).

Chlorantraniliprole 18.5% SC proved to be growth stimulative for *B. bassiana, M. anisopliae* and *L. lecanii* (Vijayasree, 2013) when incorporated in growth medium at 0.012 %, at the recommended dose and half dose chlorantraniliprole caused 35.09 and 30.82 per cent reduction in *B. bassiana* respectively (Joshi *et al.,* 2018). Chlorantraniliprole 0.4% G at recommended dose caused 38.21 per cent inhibition in *M. anisopliae.* (Lavanya and Matti, 2020).

Flubendiamide 39.35% SC 0.005 % resulted in 13.25 to 39 per cent inhibition in the growth of *L. lecanii, B. bassiana* and *M. anisopliae* (Vijayasree, 2013). In *M. anisopliae* growth inhibition of 37.10 per cent was observed in the medium poisoned with flubendiamide 20% EC (30 g ai ha^{-1}) (Tekam *et al.,* 2018).

2.1.1.1.2 Sporulation

Imidacloprid 700 WDG at the recommended dose, was found to be stimulative to the sporulation of *M. anisopliae* and *Paecilomyces* sp. as it increased the spore count by 11.32 and 28.7 per cent, while it was inhibitive (7.34 per cent) to *B. bassiana* (Neves *et al.,* 2001). Varied response to imidacloprid 17.8% SL 0.06 % was observed in two strains of *B. bassiana,* where it produced more conidia in BbDc34 and less in BbYs 35 (Gonzalez and Pena, 2017).

Thiamethoxam 25% WG 0.005 % inhibited the sporulation of *B. bassiana* and *M. anisopliae* by 12.37 and 22.32 per cent respectively. The same concentration was found to increase the spore count in *Paecilomyces* sp. by 43.43

per cent (Neves *et al.,* 2001). Thiamethoxam 250 WG 0.02% enhanced the sporulation *L. lecanii* by 45 per cent (Filho *et al.*, 2001).

Chlorantraniliprole 18.5% SC at 0.03 % resulted in medium sporulation of *M. anisopliae* strains, whereas at 0.025 % the spore count was high (Johnson *et al.,* 2020).

2.1.1.1.3 Germination

Imidacloprid 17.8% SL 0.003 to 0.006 % inhibited the spore germination of *L. longisporum* by 81 to 90.5 per cent (Panahi *et al.,* 2012). Tolerance to imidacloprid 17.8% SL among the various *B. bassiana* isolates varied in terms of germination from 60 to 92.45 per cent, at recommended dose. Isolate B55 was most tolerant with no germination inhibition at half dose and $1/10^{th}$ the recommended dose (Usha *et al.*, 2014).

Thiamethoxam 25% WG did not cause any inhibition in *B. bassiana* and *M. anisopliae,* while it caused significant reduction in the germination of *Paecilomyces* sp. (Neves *et al.,* 2001). In *M. anisopliae* rate of germination was low, 20 h after incubation in thiamethoxam 250 WG 0.02 %, with an increase after 48 h (Silva *et al.*, 2013).

2.1.1.1.2 Old Generation Insecticides

2.1.1.1.2.1 Radial growth

Malathion 50% EC 0.1 % caused 50 per cent reduction in radial growth of *B. bassiana* and 88 per cent in *M. anisopliae* (Prabhu *et al.,* 2007).

Quinalphos 20% EC caused least growth inhibition in *M. anisopliae* (Khan *et al.,* 2012). However, in *B. bassiana* isolate B57 caused 84.13 per cent inhibition to vegetative growth (Usha *et al.,* 2014).

The medium poisoned with dimethoate 30% EC 0.05 % caused 27.81 per cent inhibition in *L. lecanii* and 21.25 per cent in *B. bassiana* (Kakati *et al.*, 2018), whereas 0.015 % was found to be non-toxic with 20 per cent reduction in growth (Kachot *et al.*, 2018).

Growth of *B. bassiana* completely inhibited by chlorpyriphos 20% EC at 0.015 % (Oliveira *et al.*, 2003). At 0.006 % and 0.06 % the percentage reduction reported in growth was 27.33 and 48.33 per cent by Ambethgar *et al.* (2009). In another study by Lavanya and Matti (2020), higher concentration of chlorpyriphos 20% EC at 0.12 % was reported to completely inhibit the growth of *M. anisopliae.*

2.1.1.1.2.1 Sporulation

Malathion 50% EC 0.1 % caused 88 per cent reduction in the sporulation of *M. anisopliae* while it was 42 per cent reduction in *B. bassiana* (Prabhu *et al.*, 2007). Quinalphos 20% EC at recommended dose caused complete inhibition of sporulation in *B. bassiana* isolate B57 (Usha *et al.*, 2014).

Dimethoate 30% EC at 0.02 % and 0.05 % was found to decrease sporulation of *B. bassiana* by seven and 34.10 per cent respectively (Raj *et al.*, 2011). Neerja and Manjula (2014) reported strong suppression (91.66 per cent) of *N. rileyi* when the concentration was 0.05 %.

Chlorpyriphos 20% EC 0.04 % completely inhibited sporulation of *Beauveria brongniartii* (Saccardo) Petch, *B. bassiana and M. anisopliae* in poisoned medium (Prabhu *et al.*, 2007). At 0.1 to 1 % concentration chlorpyriphos 20% EC was detrimental to the sporulation of *B. bassiana* (Usha *et al.*, 2014).

2.1.11.2.3 Germination

Quinalphos 20% EC 0.15 % resulted in equal level of inhibition (78.3 and 77.2 per cent) in the germination of *B. bassiana* and *M. anisopliae* (Khan *et al.*, 2012).

Dimethoate 30% EC 0.02 to 0.05 %, affected spore germination by 9 to 26 per cent in *B. bassiana* (Raj *et al.*, 2011). At 0.05 % concentration, it results in 90 per cent reduction in germination in *M. anisopliae* and *B. bassiana* (Khan *et al*, 2012).

Medium poisoned with chlorpyriphos 20% EC 0.03 % completely inhibited germination of *B. bassiana* (Oliveira *et al.*, 2003). *B. bassiana* strains Bb-11, B-13 and Bb-N was completely inhibited by chlorpyriphos 20% EC whereas only 69.38 per cent reduction was observed in Bb-5A thus indicating tolerance of later strain (Rajanikanth *et al.*, 2010). Chlorpyriphos 20% EC at field dose caused nearly 50 per cent inhibition while at double dose there was complete inhibition in *M. anisopliae* and *B. bassiana* (Abidin *et al.*, 2017).

2.1.2 Tolerance to Fungicides

2.1.2.1 New Generation Fungicides

2.1.2.1.1 Radial Growth

Azoxystrobin 23% SC 0.05 % was deleterious to the vegetative growth of *B. bassiana* (Silva and Neves, 2005). At 0.1% it was proved to be fungistatic on *B. bassiana* and *M. anisopliae* (Shah *et al.*, 2009).

Hexaconazole 5% SC at half and recommended doses completely inhibited the growth of *M. anisopliae* (Lavanya and Matti, 2020; Johnson *et al.*, 2020).

Tebuconazole 1 and 10 ppm proved to be highly toxic to *L. lecanii* with very little growth which terminated at 100 ppm (Reddy *et al.,* 2018).

2.1.2.1.2 Sporulation

Azoxystrobin 23% SC at 0.1 % reduced the conidiation by 50 per cent in *M. anisopliae* (Udayababu *et al.,* 2012). In another study Silva *et al.* (2013) reported that azoxystrobin 23% SC 0.05 % did not affect the sporulation in *M. anisopliae.*

Hexaconazole 5% SC at 0.2 % completely inhibited the sporulation of *B. bassiana* (Raj *et al.,* 2011). At a concentration of 0.1 % it completely inhibited the sporulation of *N. rileyi* (Neeraja and Manjula, 2014).

Loureiro *et al.* (2002) reported complete inhibition of sporulation in *B. bassiana, M. anisopliae, I. fumosorosea* and *L. lecanii* in tebuconazole 200 EC poisoned medium. Another formulation of 25.9% EC at 100 ppm was also found to be completely inhibitive to the sporulation of *L. lecanii* (Reddy *et al.,* 2018).

2.1.2.1.3 Germination

Germination of *M. anisopliae* and *B. bassiana* was severely inhibited by azoxystrobin 23% SC at its recommended dose with 10 and 80 per cent inhibition (Shah *et al.,* 2009). At 0.04 %, it inhibited germination of *I. fumosorosea* by 80 per cent as per the report of Alessandro *et al.* (2011).

Hexaconazole 5% EC 0.05 % was also completely inhibitive to the germination of *B. bassiana* (Raj *et al.,* 2011).

Tebuconazole 25% EC 0.1 % was found to be completely suppressing the germination of both *B. bassiana* and *M. anisopliae* (Khan *et al.*, 2012).

2.1.2.2 Old Generation Fungicides

2.1.2.2.1 Radial Growth

Carbendazim 50% WP 0.1 %, completely inhibited the growth rate of *L. lecanii* with no increase in mycelial length 14 Day After Inoculation (DAI) (Krishnamoorthy and Visalakshi, 2007). Whereas, at 0.02% it caused 93.75 per cent growth inhibition in *M. anisopliae* (Parjane *et al.*, 2020). Carbendazim 50% WP at 1 g L^{-1} completely inhibited the colony growth of *M. anisopliae* (Johnson *et al.*, 2020).

Gonzalez *et al.* (2012) categorized *L. lecanii* as lightly toxic, as the medium poisoned with mancozeb 75% WP 10 mg kg^{-1} to 2000 mg kg^{-1} caused 6.2 to 34.3 per cent inhibition. In *B. bassiana* it caused higher inhibition (44.8 to 49.16 per cent) at 0.25 to 0.5% (Joshi *et al.*, 2018).

Copper oxychloride 50% WP 0.2 % caused 79.24 per cent inhibition in the growth of *L. lecanii* as observed by Olan and Cortez (2003). At 0.05 to 0.2 %, it was found to be least toxic to *B. bassiana* when compared with other copper-based fungicides (Martins *et al.*, 2012). When applied at 2.5 g L^{-1} it resulted in 47.50 per cent inhibition of *M. anisopliae* (Parjane *et al.*, 2020). There was 22.4 and 28.9 per cent inhibition in *B. bassiana* and *M. anisopliae* when tested at 0.2 % (Shoeb *et al.*, 2021).

2.1.2.2.2 Sporulation

Carbendazim 50% WP totally inhibited sporulation of *M. anisopliae.* (Rachappa *et al.*, 2007). It resulted in complete inhibition of *B. bassiana, B. brongniartii* and *M. anisopliae* (Prabhu *et al.*, 2007). They also reported that mancozeb 80% WP at 0.08 % caused 85.7 per cent and 69.5 per cent reduction in *B. bassiana* and *M. anisopliae*. Mancozeb 75% WP 2000 mg kg^{-1} was recorded as lightly toxic to *L. lecanii* by Gonzalez *et al.* (2012) as there was no exponential reduction in sporulation.

Copper oxychloride 50% WP resulted in 55 per cent reduction in conidiation of *B. bassiana* (Rachappa *et al.*, 2007). At 1% concentration it reduced the spore count in *B. bassiana* isolates B44, B56 and B57. (Usha *et al.*, 2014).

2.1.2.2.3 Germination

According to Majchrowicz and Poprawski (1993) copper oxychloride 50% WP 1 % completely inhibited the conidial viability of *M. anisopliae* and *B. bassiana.* At 0.05 % it caused 69 per cent inhibition in the germination of *M. anisopliae* (Vidhate *et al.*, 2015) and 0.2 % completely inhibited the germination of *B. bassiana* and *M. anisopliae* (Shoeb *et al.*, 2021).

Carbendazim 50% WP at 0.2 % caused cent per cent inhibition in the viability of *L. lecanii* and *B. bassiana.* (Krishnamoorthy and Visalakshi, 2007; Challa and Sanivada, 2014).

In a study conducted by Ali *et al.* (2013) mancozeb 75% WP completely inhibited conidial viability of *Lecanicillium muscarium* (Petch) Zare & W. Gams. At 0.001 to 0.01 % concentration, it caused 13 to 45 per cent reduction in germination of *B. bassiana.*

2.1.3 Artificial Selection for Pesticide Tolerance

Artificial selection and the discovery of strains that naturally exhibit desirable characteristics are easier ways to improve potential of biocontrol agent (Gaugler, 1987; Hoy, 1986). Artificial selection has been used to develop pesticide resistance in entomopathogenic fungi such as *B. bassiana* and *Metarhizium brunneum* Petch (Shapiro-Ilan *et al.*, 2002; Shapiro-Ilan *et al.* 2011; Joseph, 2014; Nilamudeen, 2015). Perusal of literature does not reveal any attempts in the genus *Lecanicillium.*

Molecular techniques, particularly transformation to benomyl resistance, have been used to increase fungicide resistance in Hyphomycetes fungi such as *B. bassiana* and *M. anisopliae* (Bernier *et al.*, 1989; Goettel *et al.*, 1990). Although there were attempts of strain selection in these fungi, they were mainly for improving virulence. Only a few attempts have been made so far to induce pesticides tolerance in entomopathogenic fungi.

Artificial selection for pesticide tolerance in entomopathogenic fungi was first attempted by Shapiro-Ilan, *et al.* (2002). They studied tolerance of seven *B. bassiana* strains against fungicides such as triphenyltin hydroxide, dodine and fenbuconazole. Ten passages in highest tolerant dose resulted in improved fungicide resistance of *B. bassiana* GHA strain. When the selection pressure was removed for some strains for three generation there was no loss of enhanced resistance observed indicating the stability of induced resistance.

Shapiro-Ilan *et al.* (2011) improved fungicide resistance in *M. brunneum* and *B. bassiana* for two fungicides, fenbuconazole and triphenyltin hydroxide, by artificial selection. For both the fungi, fenbuconazole resistance was obtained both in solid and liquid media, but triphenyltin hydroxide resistance was noted only in *B. bassiana* grown in solid media.

Joseph (2014) reported that artificial selection has increased the tolerance of *B. bassiana* 32 times to the field dose of imidacloprid 17.8% SL, 16 times the field dose of carbendazim 50% WP and carbosulfan 25% EC and eight times the field dose of chlorpyriphos 25% EC, lambda cyhalothrin, mancozeb 75% WP and malathion 50% EC after ten passages through respective poisoned media.

Nilamudeen and Sudharma (2021) reported that *B. bassiana* (Bb5) and *M. anisopliae* (Ma 4) tolerated four and eight times higher the field dose of acephate 75% SC, chlorantraniliprole 18.5% SC and thiamethoxam 25% WG respectively after artificial selection for 10 generations.

2.2 THERMOTOLERANCE

Ambient temperature of most of the agroecosystems during the growing season ranges from 10 °C to 40 °C. Entomopathogenic fungi prefer an optimum temperature between 25 °C and 30 °C (Ekesi *et al.*, 1999; Maniania *et al.*, 2008). Different fungi vary in their tolerance to varied temperature regimes which may or may not alter their growth, sporulation or germination.

2.2.1 Effect of Temperature on Mycelial Growth

Laboratory studies on temperature tolerance of *L. lecanii* revealed that growth occurred between 7 °C and 32 °C, where the optimum temperature was 23 °C to 28 °C and growth terminated above 34 °C (Li *et al.*, 1991). Llorca and Carbonell (1999) tested eight *L. lecanii* strains and found that 25°C was the best temperature for growth and at 40 °C none of the strains could grow. At 4°C and 7 °C, growth was significantly reduced in comparison to warmer temperatures. According to Monteiro *et al.* (2005) growth of *L. lecanii* was favored by the temperature 19 °C, 22 °C and 25 °C which was significantly different from the growth noted at 28 °C.

Hong and Kim, (2007) reported that *Lecanicillium* spp. exhibited optimum growth at 25 °C. Kope *et al.* (2008) studied the effect of temperature on growth of one strain of *L. longisporum*, 17 strains of *L. muscarium* and three strains of *Lecanicillium pissodis* Kope & I. Leal. The results revealed that at all the tested isolates grew between 5 °C and 30 °C, with evidence of retardation in growth after 30°C. Rate of growth increased from 0.21 to 1.86 mm day^{-1} in *L. longisporum*, 0.22 to 2.03 mm day^{-1} in *L. muscarium* and 0.21 to 1.97 mm day^{-1} in *L. pissodis*.

In an experiment conducted by Tefera and Pringle (2003) *M. anisopliae* had highest radial growth at 30 °C (3.5 cm) and lowest radial growth at 15 and 35 °C. A study to evaluate conidial thermotolerance of *M. anisopliae* isolated

from difference geographical origins recorded three isolates that could tolerate temperature between 41 °C and 49 °C for 4 h of exposure (Rangel *et al.*, 2005).

M. anisopliae growth decreased sharply with increase in temperature to 34 °C (1.2 mm day $^{-1}$). At 37 °C, it showed rosette like growth and it was arrested at 40 °C (Mustafa and Gaur, 2008). Colony growth of the 18 fungal isolates of *M. anisopliae* on SDAY plates showed different patterns when exposed to temperature regime of 10 °C to 35 °C. Of those, nine did not grow at 10 °C, three not at 15 °C and six not at 35 °C. For seven of the tested isolates optimum temperature was 25 °C (4.6 ± 0.7 mm d^{-1}) and the rest grew best at 30 °C (3.60 ± 0.7 mm d^{-1}) (Li and Feng, 2009).

Comparison of *M. anisopliae*, *Metarhizium acridum* and *Metarhizium robertsii* at different temperature regimes revealed that rapid mycelial growth occurred between 28°C and 32°C. At 38°C and above the growth was completely inhibited in all the species (Rangel *et al.*, 2010). Growth of *M. anisopliae* occurred between 15 °C and 35 °C but it was terminated at 10 °C and 40 °C (Kuboka, 2013).

Teja and Rahman, (2016) studied the thermotolerance of various *M. anisopliae* isolates and found that its maximum growth was at 30 °C followed by 25 °C. At 35 °C, growth was retarded in all the isolates and none on the isolates grew at 40 °C to 45 °C.

Tefera and Pringle (2003) reported that *B. bassiana* isolates had highest growth at 25 °C (2.8 cm) and its growth ceased when exposed to 35 °C. Devi *et al.* (2005) studied the effect of temperature cycle with 8 h of high temperature (32, 35, 38 and 42 °C) and 16 h of lower temperature (25 °C) on 29 isolates of *B. bassiana* and found that all the isolates showed more than 90 per cent relative growth at 32 ±1°C (8 h) or 25±1°C (16 h).

According to Bugeme *et al.* (2008) growth of *B. bassiana* and *M. anisopliae* was slower at 20 °C and 35 °C than at 25 °C and 30 °C.

B. bassiana grew faster at lower temperatures of 23 °C to 27 °C, whereas *M. anisopliae* grew faster at higher temperatures above 30 °C. At 35 °C, only *M. anisopliae* isolates managed to grow. None of the isolates of both species grew at 38°C (Nussenbaum *et al.,* 2013). Borisade and Magan (2014) studied the effect of temperature on *B. bassiana, M. anisopliae, I. fumosorosea and Isaria farinosa* (Wize). All the species grew best at 25 °C except few strains which grew best at 30 °C. It was also found that *M. anisopliae* had wider tolerance range up to 37 °C and *Isaria* strains were more sensitive to narrow temperature profile.

N. rileyi had optimum growth and sporulation between 20 °C and 30 °C, with no growth below 15 °C and no sporulation above 30 °C and this has limited the epizootics potential of this fungus (Ignoffo *et al.,* 1976). Kiewnick, 2006 noted that optimum temperature for *Paecilomyces lilacinus* (Thom.) Samson was between 24 °C and 30 °C beyond which growth decreased drastically and ceased at 36°C

2.2.2 Effect of Temperature on Sporulation

The optimum temperature for sporulation of *L. lecanii* was 23 °C to 25 °C and sporulation terminated at 34 °C (Li *et al.*, 1991). Highest conidial production of 2 x 10^8 spores mL^{-1} was noted at 28 °C in *L. lecanii.* Thereafter at 32 °C the sporulation decreased to 1x 10^5 spores mL^{-1} (Rivas *et al.* 2013).

Kuboka (2013) reported that in *M. anisopliae* optimum temperature for sporulation was between 15 °C to 35 °C and no sporulation was observed at 10 and 40 °C . The highest conidia production of 10^{10} conidia mL^{-1} occurred at 25°C, while the lowest of 10^9 conidia mL^{-1} occurred at 15 °C.

Teja and Rahman (2016) while studying the effect of temperature on sporulation of *M. anisopliae* revealed that highest sporulation of 1.8 x 10^7 spores mL^{-1} was observed at 30 °C followed by 6.06 x 10^6 spores mL^{-1} at 25 °C, while it failed to sporulate at 40 °C and 45 °C.

According to Tefera and Pringle (2003), the optimum sporulation temperature is 20 °C for *B. bassiana* and 25 °C for *M. anisopliae*. Nussenbaum *et al.* (2013) while studying the response of *B. bassiana* and *M. anisopliae* isolates to temperature noted that the isolates sporulated at 23 °C, 27 °C and 30 °C, while at 35 °C only two isolates Bb23 and Bb301 could sporulate.

2.2.3 Effect of Temperature on Conidial Germination

Germination of *L. lecanii* isolate HRI1.72 was faster compared to *B. bassiana M. anisopliae* and *Paecilomyces fumosoroseus* (Wize) at 10 °C as per the report of Yeo *et al.* (2003). Dimbi *et al.* (2004) reported that germination of *M. anisopliae* was highly affected at lower temperature of 15 °C, where germination was below five per cent. In *M. anisopliae* rate of germination increased with increase in temperature from 15 to 30 °C, which declined thereafter.

Germination was 100 per cent at 25 °C and the lowest (4.2 per cent) at 15 °C. At 35 °C, germination declined to 7.30 per cent. (Kuboka, 2013). The conidia of the *M. anisopliae* germinated at 25 °C and no germination was observed at 40 °C and 45 °C even after 72 h of incubation in exposed temperature (Teja and Rahman, 2016).

In the thermotolerance study conducted by Rangel *et al.* (2010), it was found that *M. acridum* isolate ARSEF 324 that exhibited 30 per cent germination at 38°C was the most thermotolerant isolate.

According to Devi *et al.* (2005), among the 29 isolates of *B. bassiana*, only one isolate showed more than 75 per cent germination at 42 °C, while at 25 °C all the isolates exhibited more than 90 per cent germination.

In the study conducted by Mustafa and Gaur (2008) it was found that the rate of germination in *B. bassiana* isolates was faster at 28 °C and all the conidia germinated at this temperature. Germination of *M. anisopliae* and *B. bassiana* was severely inhibited at 15 °C (below 10 per cent) and slightly inhibited at 35 °C

(80 to 85 per cent). All the isolates had more than 85 per cent germination when tested at 25 and 30 °C.

Germination of all the tested isolates of *B. bassiana* and *M. anisopliae* ranged between 65 to 85.6 per cent at 20 °C to 35 °C (Bugeme *et al.*, 2009). The optimum temperature for germination was 27 °C for *M. anisopliae* and *B. bassiana*. *M. anisopliae* germinated at 35 °C with high germination rate of 96.4 to 100 per cent, while *B. bassiana* isolates failed to germinate at this temperature (Nussenbaum *et al.*, 2013).

Spore suspensions of *B. bassiana*, *M. anisopliae* and *P. fumosoroseus* exposed to wet heat at 40, 42, 45 and 50 °C, for different exposure periods of 15, 30, 45 and 60 min revealed that the isolate of *M. anisopliae* thrived better with 100 per cent germination at 30 to 35 °C and 9.4 per cent germination at 45 °C (Hsia *et al.*, 2014).

2.2.4 Artificial Selection of Thermotolerant Strain

Devi *et al.* (2005) studied temperature tolerance of 29 isolates of *B. bassiana* for their ability to germinate and grow in different temperature regimes. An isolate ARSEF 2860 that showed 80 per cent relative growth at 38 °C was found to have thermal threshold above 43 °C was selected as the thermotolerant strain.

De crecy *et al.* (2009) developed an automated continuous culture method called Evoluagator™, which took over a four month time course, 22 cycle of dilution and growth to develop thermotolerant variant of *M. anisopliae*. Two variants displayed robust growth at 36.5 °C and one among that was able to tolerate 37 °C temperature.

Thermotolerance can be obtained by growing entomopathogens on different substrates. *I. fumosorosea* SFP-198 (KCTC 0499BP) grown on crushed corn had most thermotolerant conidia compared to that grown in yellow soybean, red kidney bean and rice. In *B. bassiana* and *M. anisopliae* conidia grown on

millet grain had thermotolerance at 43 to 47 °C than that those grown on Potato Dextrose Agar Yeast media (PDAY) (Kim *et al.*, 2010).

Avanti *et al.* (2014) isolated temperature mutants of *B. bassiana* and *L. lecanii* to moist heat which yielded higher biomass and blastopore at 27 and 35 °C as compared to wild type.

Hasan (2016) developed five chlorate resistant mutants of *L. lecanii* which had faster vegetative growth at different temperatures than its wild type and the wild type was less virulent than the mutants. Rate of germination of spores from mutants of *L. lecanii* was faster and exhibited thermotolerance with respect to conidial germination as compared to that of wild type.

2.3 UV TOLERANCE

The deleterious effect of solar radiation limits survival and dispersal of fungi and also deter the use of entomopathogens in biological control of crop pests (Fargues *et al.*, 1996)

2.3.1 Effect of UV Spectrum on Fungi

The UV spectrum is divided in to three wavelengths: UVA (315 to 400 nm), UVB (280 to 315 nm), and UVC (100 to 280 nm) (Coblentz, 1932). UVA and UVB rays reach the earth as ozone layers reduces the penetration of rays with wavelengths shorter than 320 nm and completely excludes wavelength below 290 nm. (Caldwell and Flint, 1997). But it is evident that UVA radiation is far less efficient in producing direct photo-lesions than UVB (Schuch *et al.*, 2009; Cadet *et al.*, 2012, 2015).

UVA and UVB components of sunlight are the major detrimental factors for fungal propagules sprayed on leaf surface. It is the main reason for short persistence of mycoinsecticides in epigeal habitat (Hughes *et al.*, 2003).

UVA represents about 95 per cent of total solar UV and is associated with conidial death and delayed germination of entomopathogenic fungi (Braga *et al.*,

2001a). But the UVB component is considered as more damaging and has been the general focus in most of the studies (Braga *et al.*, 2001b).

UVB appeared to be the most detrimental part of natural radiation, although UVA was also harmful on *P. fumosoroseus* and *M. anisopliae* (Fargues *et al.*, 1997 and Braga *et al.*, 2001b).

2.3.2 Screening for UV Tolerance

Selection of UV tolerant strains has many constraints as numerous conditions interferes with the assessment of conidial tolerance of entomopathogenic fungi to UV light. Nutrient availability, the exposure of fungi to visible light after irradiation, the age of fungal cultures is some among them (Fernandes *et al.*, 2015). Therefore, UV tolerance of the fungi can be assessed based on their germination percentage, growth and sporulation.

2.3.2.1 Effect of UV irradiance on Colony Growth

Braga *et al.* (2001b) studied the effect of UVB on colony growth of *M. anisopliae* after 48 h of treatment. The results revealed that there is significant reduction in colony growth with increase in exposure duration. The mean colony size of non-exposed colony was 2.65 mm, while it was 1. 22 mm in 1 h exposure and 0.77 mm, in 2h exposure.

Exposure to UV radiation continually depleted the growth with escalated exposure duration. Growth rate was 1.84 mm day $^{-1}$ in untreated control, while it was 1.55 mm day^{-1} at 60 min exposure (Cagan and Svercel, 2001).

2.3.2.2 Effect of UV Irradiance on Sporulation

Among two isolates of *B. bassiana* (AT076 and AT007), growth of AT076 was found to be greater than that in AT007 at 20 °C and 30 °C. It was also observed that the isolate AT076 had more spore yield of 1.61×10^7 spore disc^{-1} at 30°C and 44.33 per cent germination after UVC radiation for 15 min (Ortucu and Algur, 2017).

2.3.2.3 Effect of UV Irradiance on Germination

Exposure to UVA impacted the relative culturability of *M. anisopliae.* Culturability was not affected with one or two hour exposure, but with three and four hours there was 35 and 80 per cent decrease (Braga *et al.,* 2001b).

Fargues *et al.* (1997) observed that three hours exposure to UVA resulted in 90 per cent inhibition in *P. fumosoroseus.* Braga *et al.* (2001b) reported that *M. anisopliae* conidia exposed to UVA, was not inhibited when the exposure duration was one or two hours, but long exposure for three and four hours caused 20 and 40 per cent inhibition.

Isolates of *I. fumosorosea* from warmer regions were more tolerant to one hour UV exposure than isolates which were from temperate regions (Fargues *et al.,* 1996). Braga *et al.* (2002) evaluated the effects of UVB radiation on culturability and germination of *L. lecanii* and *Aphanocladium album* (Preuss) Gams. Increase in UVB exposure decreased relative culturability of both fungi and four hour exposure to UVB resulted in zero culturability.

Among *B. bassiana* isolates, the one isolated near lower latitudes had higher UVB tolerance than those from higher latitudes due to evolutionary adaptation to higher irradiance near equator compared to higher latitudes, where the irradiance is much lower (Fernandes *et al.,* 2007).

Impact of culture age of on UVB tolerance (λ 312 nm) was studied by Moore *et al.* (1993) where it was seen that the conidia from older cultures of *M. acridum* were more tolerant to UV irradiance than younger conidia. In another experiment on *B. bassiana*, *M. anisopliae*, *L. lecanii* and *L. muscarium*, it was revealed that irradiated conidia of all the four fungi germinated at lower rate when compared to non- irradiated conidia, however this reduction in germination (20 to 80 per cent) was unaffected by the culture age (Le Grand and Cliquet, 2013).

Mustafa and Gaur (2008) studied effect of UVB radiation on germination of *M. anisopliae* and *B. bassiana*. Out of 14 isolates of *M. anisopliae* only three isolates showed 100 per cent germination with four hour UVB exposure and six isolates showed less than 30 per cent germination. In *B. bassiana* among 17 isolates, seven of them had more than 95 per cent germination when exposed for four hours to UVB.

UV tolerance varies with the different phases of germination in *M. acridum* and *M. robertsii*. The tolerance increased during the first four to six hours of germination, followed by pronounced decrease in tolerance as germination proceeded (Braga *et al.*, 2001b).

Braga *et al.*, 2002 reported that germination of *L. lecanii* was not affected after one hour of UVB exposure, while three to four hour exposures, reduced the germination to 40 and 20 per cent, respectively.

UVB radiation delayed and decreased the germination of *M. anisopliae* spores. One hour exposure led to 12 per cent germination and this value rose to 95 per cent after 48 h (Braga *et al.*, 2001a). In *Beauveria* spp. there was only 9.5 per cent germination at 24 h while the rate rose to 30 per cent after 48 h. (Fernandes *et al.*, 2007).

Tolerance of 56 isolates of *Beauveria* spp. isolated from different latitude, to UVB radiation when studied, variable tolerance was noted in terms of germination. Germination varied between 0 to 80 per cent and the isolate CG228 had highest tolerance (Fernandes *et al.*, 2007).

B. bassiana isolate Bb 259 under UVB (λ 312 nm) exposure exhibited 100 per cent germination after one and two hours of exposure and 75 per cent germination after four hour exposure (Posadas *et al.*, 2012).

The UVB tolerance of *Tolypocladium cylindrosporum* Gams, *Tolypocladium geodes* Gams and *Tolypocladium inflatum* Gams exposed to UVB for one, two, three and four hours was studied in comparison with *M. robertsii*.

After three hour exposure, *T. cylindrosporum* germinated at a greater rate (80 per cent) than the other species (15 to 20 per cent) and had similar viability to that of the *M. robertsii* (Santos *et al.*, 2011).

Rodrigues *et al.* (2016) studied the effect of UVB on conidial germination of *B. bassiana* and *M. anisopliae* for exposure durations varying from five minutes to 30 minutes and found that in *B. bassiana* there was 52, 11 and one per cent germination when exposed to five, 10 and 15 min respectively. Whereas, in *M. anispliae* there was 64 per cent germination only with five min exposure and with 15 min exposure there was only one per cent germination. In both the fungi germination terminated with 30 min exposure.

Couceiro *et al.* (2021) reported that the germination of conidia was not affected in *M. anisopliae*, *M. robertsii* and *M. brunneum* after two hour exposure to UVB. After 4 hours of exposure there was 70 per cent germination. It started to decline after six hour exposure and reached five per cent after eight hour exposure.

The effect of UVB on colony size of *Metarhizium* spp. was significant at six hour exposure. *M. brunneum* showed higher growth inhibition (28.9 per cent) than *Metarhizium guizhouense* QT Chen & HL Guo and *M. robertsii* (23.7 per cent and 28.9 per cent) (Fernandez-Bravo *et al.*, 2017).

2.3.2 Induction of UV Tolerance

Zhao *et al.* (2016) isolated UV mutant of *M. anisopliae* by exposing to UVC radiation and designated it as MaUV-40.1 which displayed both more rapid growth and increased virulence. The LT_{50} value of 10^6 conidia mL^{-1} of *M. anisopliae* on *Plutella xylostella* L. was 57.6 and 115.4 h for the mutant and wild-type strains, respectively.

Exposure of *B. bassiana* to UV rays increased the infectivity of it on *Ostrinia nubilalis* (Hubner). The mutant obtained at 45 min of UV exposure showed highest level of infectivity. UV exposure had detrimental effect on the

radial growth of the fungus and it was found to decrease with increase in UV exposure. (Cagan and Svercel., 2001).

Exposing *B. bassiana* to UV rays for 30 and 60 min increased its virulence on *Chilo agamemnon.* Bles. The LC_{50} values of mutants obtained at 30 min and 60 min exposure ranged between 112 to 179 spores mL^{-1} and 144 to 181 spores mL^{-1} respectively, whereas in wild type the LC_{50} was significantly higher (221 to 240 spores mL^{-1} (Sahab *et al.,* 2014).

2.4 REPETITIVE EXTRAGENIC PALINDROMIC ELEMENT (REP) PCR ANALYSIS

Microbial genome comprises a variety of repetitive DNA sequences, accounting for up to five per cent of genome (Ussery *et al.,* 2004). Some of these repetitive elements may be involved in catabolism of DNA and RNA (Tobes and Ramos, 2005), or to be mediators of genome evolution (Schmidt and Anderson, 2006).

Interspersed repeated elements range in size from 15 base pairs to hundreds of base pairs. Among these repetitive elements, the Repetitive extragenic palindromic (REP) elements which are about 40 base pairs long are potential regulatory sequences within untranslated regions of operons by virtue of their palindromic nature and ability to form stable stem-loop structures in transcribed RNA. Therefore, REP-PCR DNA fingerprinting methodology is a simple and fast method with sufficient resolving power for subspecies or strain identification (Versalovic *et al.,* 1998).

REP-PCR method has also been used for evaluation of genetic diversity of fungi including *Fusarium oxysporum* Schlecht, *Verticillium dahlia* Kleb (Komatsu *et al.* (2001) and *Rhizoctonia solani* Kuhn.

Abbasi *et al.* (2016) used REP primers to differentiate mutants in *Trichoderma harzianum* Rifai, where gamma induced mutants were

differentiated using REP-PCR primer, rep1R-I. It produced more polymorphic bands and detected more loci than other rep primers.

Soufi *et al.* (2021) revealed that UPGMA analysis of REP-PCR fingerprints in *Trichoderma aureoviride,* Gamma induced mutants varied 70 per cent from the wild type culture.

Trombert *et al.* (2007) revealed that REP - PCR could be used to study UV induced changes on DNA in bacteria and the use of a single pair of primers with a unique PCR reaction is enough to differentiate strains present in UV treated samples. They concluded that REP - PCR appears as a rapid, robust, useful complementary methodology to monitor the impact of UV irradiation at molecular level.

Joseph (2014) revealed that RAPD analysis of carbendazim tolerant strain of *B. bassiana* Bb5 and *M. anisopliae* Ma 4 exhibited 83 per cent and 38 per cent polymorphism, compared to their mother culture. Whereas, in the report of Nilamudeen (2015) observed no molecular variation in RAPD analysis of insecticide tolerant cultures of the same isolate except minor polymorphism of 1.61 per cent exhibited in *B. bassiana.*

2.5 PATHOGENICITY OF TOLERANT / SELECTED STRAINS

Shapiro-Ilan *et al.* (2002) reported that artificial selection for fungicide tolerance did not affect the virulence of GHA strain to pecan weevil, *Curculio caryae* Horn, whereas fungicide exposure increased virulence in a mixed wild population of *B. bassiana.*

Shapiro-Ilan *et al.* (2011) reported that artificial selection caused negative impact on the virulence of *B. bassiana,* where non-selected population exhibited higher virulence on *Galleria mellonella* L. than the selected population. They concluded that selection for fungicide resistance can result in unpredictable impacts which are positive or negative on other traits.

Zhao *et al.* (2016) reported that UV mutants of *M. anisopliae* exhibited higher virulence on diamondback moth, *P. xylostella*. The LT_{50} using 10^6 conidia mL^{-1} was 57.6 h and 115.4 h for the MaUV- 40.1 mutant and wild-type strains, respectively.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Tolerance of entomopathogenic fungi *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and *Lecanicillium lecanii* (Zimm.) Zare and Gams to abiotic stress" was carried out in the Biocontrol Laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram during the year 2017-2021.

L. saksenae and *L. lecanii* cultures maintained in the Biocontrol laboratory, Department of Agricultural Entomology, College of Agriculture, Vellayani were utilized for the study. *L. saksenae* (Accession no: ITCC LsVs-1-7714) is an indigenous isolate from the soils of Vellayani isolated by Rani *et al.* (2014) and *L. lecanii* isolate No. V1 8, was originally sourced from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. The cultures of *L. saksenae* and *L. lecanii* were revived by passing through *Leptocorsia orotarius* (Thunberg) and brinjal mealy bug *Coccidohystrix insolita* Green respectively. Pure cultures were maintained Potato Dextrose Agar (PDA) slants and mass multiplied in Potato Dextrose Broth (PDB).

3.1 SCREENING FOR TOLERANCE TO INSECTICIDES

Tolerance of *L. saksenae* and *L. lecanii* to commonly used insecticides were carried out under *in vitro* conditions using poisoned food technique. It was assessed based on growth in terms of mean colony diameter, sporulation by counting the number of conidia using a Neubauer Haemocytometer and germination by counting the number of germinated spores under 40x magnification in a compound microscope (Motic BA 210).

3.1.1 Tolerance to Insecticides

Tolerance of the fungi was tested at three different regimes, the recommended dose, half the recommended dose and double the recommended dose of each of the insecticide listed in the Table 1. The insecticides selected for the study included old as well as new generation ones as detailed below. The experiment was laid out in Completely Randomised Design (CRD) with 24 treatments each replicated thrice with two plate per replication.

Table 1. Insecticides screened for tolerance

Sl. No.	Insecticide		Dose (%)		
	Chemical name	Trade name	Recommended	Half	Double
1.	Malathion 50% EC	Riddle	0.1	0.05	0.2
2.	Quinalphos 25% EC	Ekalux	0.05	0.025	0.1
3.	Dimethoate 30%EC	Tafgor	0.04	0.02	0.08
4.	Chlorpyrifos 20% EC	Chlorguard	0.06	0.03	0.12
5.	Flubendiamide 39.35% SC	Fame	0.005	0.0025	0.01
6.	Chlorantraniliprole18.5%SC	Coragen	0.006	0.003	0.012
7.	Imidacloprid 17.8% SL	Confidor	0.006	0.003	0.012
8.	Thiamethoxam 25% WG	Actara	0.005	0.0025	0.01

The data were subjected to analysis of variance (ANOVA) using WASP 2 software and treatment variations were related. Those insecticides which were found to be non-compatible were selected for further studies on artificial selection.

3.1.1.1 Growth Bioassay

To prepare poisoned medium double strength PDA was prepared. The medium was sterilized in a vertical autoclave at 121 °C at 1.1 kg cm^{-2} for 20 min. The required quantity of pesticide was dissolved in sterile double distilled water

which was added to equal quantity of molten double strength PDA. This poisoned PDA (15 mL) was immediately poured in to Petri plates for solidification. After solidification, Petri plates were inoculated with five mm disc of seven day old actively growing culture of each of the fungi, using a flame-sterilized cork borer. The Petri dishes were incubated at room temperature for the growth of inoculated fungus. PDA without pesticides served as control. Observations were recorded on growth on the seventh and 14^{th} day after inoculation (DAI) for *L. saksenae* and 10^{th} and 21^{st} DAI for *L. lecanii*. The radial growth was observed for longer duration in *L. lecanii* as it was slower growing than *L. saksenae* which was able to cover 9 cm Petri plate in 14 DAI.

3.1.1.2 Spore Enumeration

Spore count was determined from 21day old cultures. Conidia of each of the fungi were dispersed in sterile water (10 mL) with 0.02% tween 20 by scraping off the mycelia by a sterilized L rod. The suspensions were then filtered using double-layered muslin cloth. 10 µL each of the suspensions were transferred into the haemocytometer using a micropipette for counting the spores.

3.1.1.3 Conidial Germination

Spore suspensions of the fungi were prepared from 21 day old cultures and their spore count was adjusted to 10^5 conidia mL^{-1} by serial dilution method. Sterilized glass slides were evenly coated with a drop of molten poisoned PDA and were allowed to dry in a laminar airflow. After drying 100 µL each of the spore suspensions were dropped on to a glass slide and were spread uniformly. The slides were incubated in a Petri dish lined with moistened filter paper for 24 h at room temperature. After 24 h, the slides were observed under 40x magnification in a compound microscope to count 100 spores at random and the number of germinated spores were noted. Spores with germ tube more than the diameter of the spores were considered to be germinated. Counting was done thrice at random points on the slides and the average was taken.

3.1.1.4 Compatibility of Entomopathogenic Fungi with Insecticides

Compatibility of insecticides with the entomopathogenic fungi was assessed to categorise insecticides as compatible or toxic based on computation method for Biological Index (BI) as proposed by Rossie-Zalaf *et al.* (2008).

BI = [47 x VG + 43 x SP + 10 x GER] / 100 where,

VG - Vegetative growth of the fungal colony (%) in relation to control

SP - Sporulation (%) in relation to control

GER - Conidial germination (%) in relation to control

BI values were grouped into three categories of toxicological classification *viz.,* 0 to 41 = toxic; 42 to 66 = moderately toxic; >66 = compatible.

The compatible insecticides were eliminated from further studies while the non-compatible insecticides were selected to induce tolerance for artificial selection.

3.2 SCREENING FOR TOLERANCE TO FUNGICIDES

As fungi are more sensitive to fungicides, their compatibility with fungicides was tested at recommended dose. The old and new generation fungicides selected for the study is detailed in the Table 2. The experiment was laid out in CRD with six treatments each replicated thrice.

Observations were recorded on growth, sporulation and conidial germination as described in Para 3.1.1.1, 3.1.1.2 and 3.1.1.3.

The compatibility of fungicides with entomopathogenic fungi was assessed as described in para 3.1.1.4. Those fungicides which were found to be non-compatible were selected to induce tolerance for artificial selection.

Table 2. Fungicides screened for tolerance

Sl. No	Chemical Name	Trade name	Recommended Dose (%)
1	Copper oxychloride 50% WP	Blitox	0.2
2	Mancozeb 75% WP	M-45	0.3
3	Carbendazim 50% WP	Bavistin	0.2
4	Hexaconazole 5% EC	Contaf 5 E	0.2
5	Tebuconazole 25% EC	Folicur	0.2
6	Azoxystrobin 23% SC	Amistar	0.1

3.3 ARTIFICIAL SELECTION FOR INSECTICIDE TOLERANCE

In order to induce tolerance to incompatible insecticides, the method suggested by Shapiro *et al.* (2002) was followed with some modifications.

3.3.1 Highest Tolerant Dose

As the growth was observed at recommended dose of insecticides the tolerance was tested starting from the recommended dose 1X with subsequent test doses 2x, 3X *etc.* until the growth was arrested. The highest concentration of the insecticides that would support atleast a minimal level of growth, germination and sporulation of the fungi was determined as Highest Tolerant Dose (HTD).

3.3.2 Induction of Insecticide Tolerance

The fungi were repeatedly sub-cultured on PDA poisoned with HTD of incompatible insecticides. The fungus thus sub cultured for 10 successive generations at HTD is termed as the "selected" culture, the one sub cultured for seven successive generations in poisoned medium and the next three generations in the unpoisoned medium is the "relaxed" culture and the normal culture is the

"non-selected" culture. The growth parameters were recorded in each successive generation.

3.3.3 Performance of Selected Strain

3.3.3.1 Phenotypic Study

To evaluate the performance of selected strain it was compared with the relaxed and non-selected cultures for their tolerance against the recommended dose of respective insecticides. The phenotypic performance was studied in terms of radial growth, germination and sporulation.

3.3.3.2 Genotypic Study

The cultures with phenotypic changes if any, was further subjected to genotypic changes using REP-PCR.

3.3.3.2.1 Cetyltrimethylammonium bromide (CTAB) DNA extraction method

DNA was isolated using CTAB DNA Extraction method (Carter -House *et al.*, 2020)

3.3.3.2.1.1 Preparation of lysis buffer

Lysis buffer consisted of three buffers Buffer A (0.35 M sorbitol; 0.1 M Tris-HCl, pH 9; 5 mM EDTA, pH 8), buffer B (0.2 M Tris-HCl, pH 9; 50 mM EDTA, pH 8; 2 M NaCl; 2% CTAB) and buffer C: 5% Sarkosyl. For two samples lysis buffer was prepared in one tube (2mL) by adding 650 µl buffer A, 650 µl buffer B, 260 µl buffer C, 175 µl 1% (v/v) PVP and 10 µl proteinase K to microcentrifuge tube, mixed then it was split equally in to two 2 mL tubes and placed in hot water bath 65 °C.

3.3.3.2.1.2 DNA isolation

About 100 mg of the mycelium was homogenized in pestle- mortar using liquid nitrogen and the powdered mycelia was transferred to a microcentrifuge tube containing lysis buffer. This mixture was incubated for 30 min at 65 °C and

mixed by inversion frequently. Then 280 µl of 5 M Potassium acetate was added to each tube and incubated on ice for 5 min. 700 µl of Phenol: Chloroform: Isoamyl alcohol (PCI), 25:24:1 was added and mixed by inversion (>5 min), incubated for 2 min at room temperature (RT). This was centrifuged at 6000 x g for 10 min and supernatant was transferred to new 2 mL microcentrifuge tube. Equal volume of Chloroform: Isoamyl alcohol, 24:1 (CI) was added, mixed by inversion for 5 min then incubated at room temperature for 2 min. and centrifuged at 6000 x g for 10 min. The supernatant was collected in 2 mL tube and 2.5ul RNase A 2.5 µl was added. After incubation for 1 h, 1/10 vol 3M sodium acetate and 1 vol of isopropanol was added, gently mixed, kept at room temperature for 5 min. The supernatant was discarded after centrifugation at 3000 x g for 2 min and precipitate was washed with 1mL freshly prepared cold 70% ethanol, centrifuged at 3000 x g for 2 min. Ethanol is poured out and pellet was dried at room temperature for 10 to 15 min until all the leftover ethanol is evaporated. The dried pellets were resuspended in 50 µl to 100 µl TE buffer at 65 °C. Finally, DNA concentration was assessed using spectrophotometer.

3.3.3.2.1.3 Agarose gel electrophoresis for DNA quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. 3 µl of 6x loading dye was mixed with 12 µl of DNA and loaded to 0.8% agarose gel prepared in 0.5X TBE (Tris-Borate-EDTA) buffer containing 0.5 μgmL^{-1} ethidium bromide. Electrophoresis was performed with 1X TBE as electrophoresis buffer at 80 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using gel documentation system (Bio-Rad).

3.3.3.2.2 Repetitive Extragenic Palindromic (REP-PCR)

REP primer used were REP 1R-I and REP 2I. PCR amplification reactions were carried out in a 25 µl reaction volume which had 12.5 µl Taq mix

(100 mM Tris-HCl; 500 mM KCl; 0.8% (v/v) Nonidet P40 and 15 mM $MgCl_2$), 10 µl any one of REP primer (40 µM), 1 µl BSA (10 ng μl^{-1}) and 1.5 µl DNA.

Primer Name	Sequence (5' → 3')	Reference
REP 1R-I	IIIICGICGICATCIGGC	Komatsu *et al.*, (2001)
REP 2I	ICGICTTATCIGGCCTAC	

DNA Thermal Cycler was programmed for initial denaturation at 94°C for 7 min, followed by 35 cycles that included denaturation at 94°C for 1 min, annealing at 40°C for 1 min, and primer elongation at 65°C for 8 min. The final elongation was at 65°C for 16 min, followed by final incubation at 10°C. PCR products were examined by electrophoresis for 70 min in 2 % agarose gel.

3.3.3.2.3 Agarose gel electrophoresis of PCR products

The PCR product were examined in 2 % agarose gels prepared in 1X TBE buffer containing 0.5 µg mL^{-1} ethidium bromide. Three µl of 6x loading dye was mixed with 12 µl of PCR products and electrophoresis was performed at 80 V power supply with 1X TBE as electrophoresis buffer for 1 to 2 h. until the bromophenol blue front had migrated to almost the bottom of the gel. The molecular standard used was 100 bp DNA ladder. The gels were visualized in a UV transilluminator and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.3.2.4 Analysis of REP-PCR profiles

To compare relatedness of strains, rep-PCR banding profiles were visually observed and amplified fragments were scored for the presence (1) or the absence (0). Calculation of the similarity between each pair of DNA fingerprints was determined by Jaccard's coefficient. Dendrograms were generated from binary data using numerical taxonomy system (NTSYS-pc) software package (version 2.1). Similarity coefficients for each pair-wise combination of REP-PCR profiles were computed by SIMQUAL (Similarity for qualitative data program)

using simple matching coefficient. Cluster analysis was performed based on the similarity coefficients using sequential agglomerative hierarchical nested (SAHN) program with an unweighted pair group arithmetic mean averages (UPGMA) algorithm. The dendrogram of the tree matrix data was constructed using the TREE (tree display) program.

3.4 ARTIFICIAL SELECTION FOR FUNGICIDE TOLERANCE

3.4.1 Highest Tolerant Dose

In those fungicides where growth was observed at recommended dose, the tolerance was tested starting from the recommended dose 1X with subsequent test doses 2X, 3X etc. until the growth was arrested. In those fungicides which would not support growth at recommended dose the dose tested ranged from $1/10^{th}$ the recommended dose (0.1X) with subsequent test doses 0.2X, 0.3X etc. until the growth was arrested. The highest concentration of the insecticides that would support atleast a minimal level of growth, germination and sporulation was determined as HTD.

3.4.2 Induction of Fungicide Tolerance

The induction of tolerance was carried out as described in para 3.3.2

3.4.3 Performance of Selected Strain

The selected, relaxed and non-selected cultures were studied for phenotypic performance and genotypic changes as mentioned in para 3.3.3.

3.5 SCREENING FOR TEMPERATURE TOLERANCE

The compatibility test carried out in insecticides and fungicides is insignificant with regard to temperature. Screening of fungi for temperature tolerance would directly indicate Highest Tolerant Temperature (HTT). Therefore, HTT of each species was determined based on growth, sporulation and germination at different temperature regimes.

3.5.1 Tolerance to Temperature

Tolerance of entomopathogens to different temperature regimes was assessed in terms of radial growth, sporulation and germination. The fungi were exposed to 8h of test temperature *viz.*, 30°C, 32°C, 34°C, 36°C, 38°C and 40°C as well as 16 h of 26°C (control) daily for 21 days in incubator. Daily temperature cycle of 8h/16 h was maintained to simulate the field conditions (Devi *et al.*, 2005).

3.5.1.1 Radial Growth

Mycelial plugs were cut from seven day old culture plates using 5 mm diameter cork borer and each agar plug were singly transferred onto the centre of a freshly prepared PDA plate. The plates were sealed with cling film and incubated in temperature regimes of 30°C, 32°C, 34°C, 36°C, 38°C and 40°C, to detect their tolerance level based on their growth, germination and sporulation. Five plates were prepared for each temperature and the experiment was repeated twice to ensure consistency of the results. Colony growth rate (mm day^{-1}) was calculated till 14th DAI for *L. saksenae* and till 21st DAI for *L. lecanii*.

3.5.1.2 Spore Enumeration

Sporulation of the fungi after exposure to different temperatures was enumerated on the 21st day as mentioned in para 3.1.1.2

3.5.1.3 Conidial Germination

Conidial germination of each of the fungi was tested at six test temperatures 30, 32, 34, 36, 38 and 40 °C at a constant temperature of 25°C (control) that were used for 8 h alternating with 16 h at 25°C. The conidia were harvested from 21 day old culture plates by flooding with sterile distilled water containing 0.02% of tween 80 and filtered through a double layered muslin cloth. Then 100 µl each of the conidial suspensions containing 10^5 conidia was spread on a slide coated with a thin film of PDA medium. The inoculated slides were kept in Petri dishes (15 cm) on a glass rod with moistened filter paper at bottom

and incubated at different temperature treatments. The spore germination was observed at 24 h and 48h after inoculation.

3.5.2 Artificial selection for Temperature Tolerance.

3.5.2.1 Highest Tolerant Temperature

Highest Tolerant Temperature (HTT) *i.e.,* the temperature which support atleast minimal growth, germination and sporulation and was determined based on the above observations.

3.5.2.2 Induction of Temperature Tolerance

The fungi were repeatedly sub cultured on PDA at HTT. The fungus thus sub cultured for 10 successive generations at HTT is termed as the "selected" culture, the one sub cultured for seven successive generations in HTT and the next three generations at 26 °C is the "relaxed" culture and the normal culture is the "non-selected" culture. The growth parameters were assessed at every generation by measuring the radial growth, spore count and germination as mentioned in para 3.5.1.

3.5.2.3 Performance of Temperature Tolerant Strain

The strains showing improved growth parameter (phenotypic changes) after subjecting to artificial selection in highest temperature were subjected to genotypic study as mentioned in para 3.3.3

3.6 SCREENING FOR UV TOLERANCE

The fungi were screened for UV tolerance by irradiating under two UV ranges UVA and UVB under two independent experiments, following the method proposed by Braga *et al.* (2002) with slight modifications.

3.6.1 Production and Preparation of Conidia for Irradiation

Each of the fungi were grown in PDA plates in dark at 26 °C for 21 days. Conidia were dispersed in sterile water (10 mL) with 0.02% tween 20 by scraping

off the mycelia using a sterile L rod. The suspensions obtained were shaken vigorously and filtered through double layered muslin cloth to remove mycelia and clumps of spores. Conidial concentration was determined with a haemocytometer. The suspensions were stored at 4 °C for 48 h before exposure.

3.6.2 Irradiation

The two cabinets were used for irradiation one was Rotek UV cabinet provided with UV tube consisting of UVA, Philips TL 8W BLB with 365 nm wavelength. Another UV cabinet was set up by installing UVB tube, Philips TL 20W/1 RS fluorescent lamp inside a laminar air flow unit which had spectral output of 312 nm. Lamps were switched on for one hour prior to the experiment to stabilize the irradiance. The effect of irradiation was studied based on colony growth, sporulation and germination.

3.6.2.1 Colony Growth

The spore suspensions of both *L. saksenae* and *L. lecanii* were exposed to three UV ranges in two UV cabinets for duration of 10, 20, 30 min, 1 h, 2h, 3h and 4h. After exposure, the spore suspension was inoculated on PDA and plates were incubated in darkness at 26 °C. The effect of irradiation on the colony growth of fungi was determined by the evaluation of the size of colonies that aroused from irradiated conidia at 48 h. Measurements were performed using a compound microscope at 40 x magnification. The mean diameter of each colony was measured based on two measurements taken at right angles. Fifty colonies per treatment were evaluated and experiment was replicated thrice.

3.6.2.2 Spore Enumeration

Sporulation of the fungi after UV exposure was enumerated on the 21st day as mentioned in para 3.1.1.2.

3.6.2.3 Conidial Germination

The experiments were conducted on PDA plates containing 10 mL media with 0.001% carbendazim. Addition of low concentration of carbendazim 50%

WP in the medium allows germination to be monitored for longer periods of time because it inhibits the growth of germ tubes without adversely affecting germination as per the method followed by Oliveira *et al.* (2015). Conidia (40 µl, 10^6 conidia mL^{-1}) were placed on the medium and immediately exposed to UV irradiance for 10, 20, 30 min, 1h, 2h, 3h and 4 h. After exposure, the plates were incubated in the dark at 26 °C. Germination was observed with 40 x magnification at 24 h and 48 h from the time when the conidia were placed on the medium. Conidia with a germ tube larger than the diameter of the conidium were considered to have germinated. A total of 100 conidia per treatment was evaluated and germination per cent was calculated

3.7 INDUCTION OF UV TOLERANCE

The highest dose among three UV radiation is UVC (254 nm) which is having germicidal property. Therefore, by using UVC wavelength an attempt was made to induce tolerance and select mutants which were UV tolerant.

3.7.1 UV mutagenesis

Fungal mutants were generated using a method described by Zhao *et al.* (2016). Conidial suspension of 5 mL with concentration of 1.0×10^6 conidia mL^{-1} was incubated at 26 ± 1 ^{0}C for 24 h in a Petri dish (9 cm diameter) in dark. These germinated conidia were exposed to UVC light for different time intervals of 0,10, 20 and 30 min. Aliquot were spread on PDA plates, and incubated in the dark at 26 ± 1°C for seven days. The colonies surviving at highest time of exposure were chosen for repeated exposure. The selected colonies were irradiated for two additional cycles *i.e.*, totally three exposure and in each round the fastest growing colonies were selected for further studies.

3.7.2 Performance of Selected Strain

3.7.2.1 Phenotypic Performance

The selected colonies were checked for UV tolerance in comparison with wild types under the two UV irradiance (UVA and UVB) at 30 min, 2 h and 4 h

of exposure. The tolerance was assessed based on growth, sporulation and germination as described para in 3.6.

3.7.2.2 Genotypic Changes

The strains were further studied for genotypic changes using REP-PCR as described in para 3.3.3.2.

3.8 PATHOGENICITY ASSAY OF SELECTED LINES OF *L. saksenae* AND *L. lecanii*

The tolerant strain obtained from previous experiments were tested for their pathogenicity on test insects. For *L. saksenae* the test insect was *Leptocorisa oratorius* Fab. and *C. insolita* was used as test insect for *L. lecanii*.

3.8.1 Pathogenicity of *L. saksenae* to *L. oratorius*

3.8.1.1 Raising Plants

Rice variety Prathyasa was used to rear the test insect rice bug, *L. oratorius*. Sprouted seeds were planted in 23 cm × 21 cm plastic pots with a 2:1:1 potting mixture of clay, soil, and cow dung. Planting was done sequentially to maintain a continuous supply of panicles during the milky stage.

3.8.1.2 Rearing of L. oratorius

Adult rice bugs were collected from the field and kept under observation for any latent infection. Plants were encased in a nylon net that was tethered to a wooden frame. Ten pairs of healthy adults were transferred to the rearing cage. Egg masses were collected by clipping the leaves. To ensure maximum emergence, these clippings were placed in a Petri dish with wet filter paper and left at room temperature for five days. Thereafter, these egg masses were transferred to the new plants and fixed at the base of panicle at milky stage using a stapler along with clippings. Plants with milky panicles were replaced every two weeks to ensure an adequate supply of food. Third instar nymphs and adults

of uniform age collected from the rearing cage were used for the laboratory studies (Valencia and Heinrichs, 1982).

3.8.1.3 Preparation of Spore Suspensions

Spore suspensions were prepared by blending the 21 day old cultures, grown in Potato dextrose broth (PDB), in a blender for 10s. The mixture was filtered in a double layered muslin cloth. Spore count was adjusted to $1x10^7$ spores mL^{-1} concentration using a Neubauer haemocytometer.

3.8.1.4 Assessment of Mortality

3.8.1.4.1 Fungicide Tolerant Strain

Pathogenicity of tolerant strain individually and in the combination with fungicide (at field concentration) was tested. For combination treatment, spore suspension and fungicide were mixed thoroughly in the ratio 1:1 and kept for an hour. Rearing jars of size 15 cm x 20 cm were taken adults were separately released into it. For each treatment 10 insects were maintained. Rice panicles with milky grains, staked in glass vials with sucrose solution were given as food. Approximately two mL spore suspensions of tolerant strain and non-selected strain was applied uniformly in the container using an atomiser. Then the container was covered with a clean muslin cloth and fastened with a rubber band. Mortality of bugs was noted at 24 h interval for a period of five days. Mortality recorded in tolerant strain and non-selected strain were compared. The experiment was laid out in Completely Randomised Design (CRD) with 6 treatments each replicated thrice with 10 insects per replication.

3.8.1.4.2 UV Tolerant Strain

The mortality of test insect by UV tolerant mutant and wild type of *L.saksenae* (Mother culture) was assessed as mentioned in Para *3.8.1.4.1.* The experiment was laid out in CRD with 3 treatments each replicated 5 times with 10 insects per replication.

3.8.2 Pathogenicity Assay of *L. lecanii* on *C. insolita*

3.8.2.1 Rearing of Mealybugs on Potato

The test insect *C. insolita* was reared on sprouted potato as described by Mani and Shivaraju, (2016). The plastic trays of dimension 13 x 14 cm were taken and filled with sterilized sand. Medium to large sized potatoes which are disease free with slight sprouts were taken and cleaned in tap water. They were planted with half part inside sand trays with 3 cm gap between tubers. Immediately after planting the trays were placed on racks in clean room and watered. The trays were watered every four or five days to get good sprouts and watering has to be stopped once the infesting is done. Ant well was constructed below the tray and to avoid ant attack. Brinjal mealybug, *C. insolita* was collected from field and released on sprouted potatoes. Once the mealybug establishes, they were used to conduct bioassay.

3.8.2.2 Assessment of Mortality

Spore suspensions of *L. lecanii* was prepared as described in para 3.8.1.3. Fresh brinjal leaves were taken in plastic Petri plate and petiole was tied with moistened cotton to avoid drying of leaves. Ten test insects were released on each leaf and the spore suspensions of UV tolerant mutant and wild type of *L. lecanii* (Mother culture) prepared in sterile water were sprayed on to the test insects using an atomiser. Mortality of insects was noted at 48 h interval for a period of eight days. The mortality of test insect by tolerant strain and non-selected strain were compared. The experiment was laid out in Completely Randomised Design (CRD) with 4 treatments each replicated 5 times with 10 insects per replication.

3.9 STATISTICAL ANALYSIS

The data obtained from the experiment were transformed as required and subjected to analysis of variance (ANOVA) using WASP 2.0 software and O P stat software.

Results

4. RESULTS

The results of the study entitled "Tolerance of entomopathogenic fungi *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and *Lecanicillium lecanii* (Zimm.) Zare and Gams to abiotic stress" carried out in the Biocontrol Laboratory, Department of Agricultural Entomology during 2017-21 is presented below.

4.1 TOLERANCE OF *L. saksenae* and *L. lecanii* TO INSECTICIDES

Tolerance of *L. saksenae* and *L. lecanii* to old generation insecticides such as malathion 50% EC, quinalphos 25% EC, dimethoate 30% EC, and chlorpyriphos 20% EC and new generation insecticides such as imidacloprid 17.8% SL, thiamethoxam 25% WG, chlorantraniliprole 18.5% SC and flubendiamide 39.35% SC, assessed in terms of radial growth, sporulation and germination is depicted below. Tolerance of fungi was assessed at half the recommended dose, recommended dose as well as at the double dose.

4.1.1 *L. saksenae*

Table 3 depicts the effect of poisoned media on the growth parameters of *L. saksenae*

4.1.1.1 Radial Growth

Radial growth of the fungus in terms of mean colony diameter (cm), recorded on seventh and 14th DAI in PDA poisoned with insecticides is presented in Plates 1 and 2.

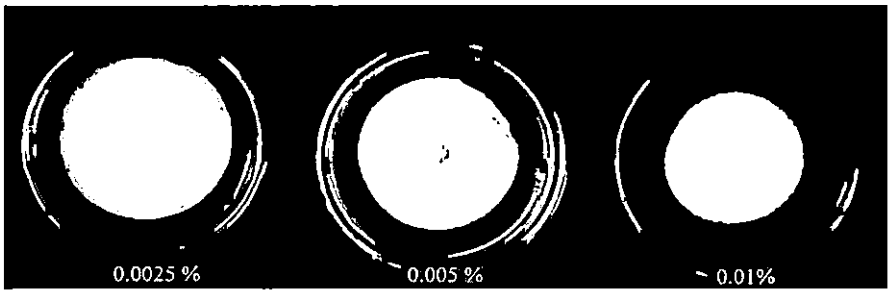
4.1.1.1.1 At Half the Recommended Dose

Analysis of data on the seventh DAI revealed that *L. saksenae* is highly tolerant to new generation insecticide, flubendiamide 39.35% SC 0.0025 % wherein the mean colony diameter was 3.30 cm. Growth in imidacloprid 17.8% SL 0.003 % was 3.27 cm followed by that in thiamethoxam 25% WG 0.0025 % and chlorantraniliprole 18.5% SC 0.003 % with no variation among them (3.23 cm).

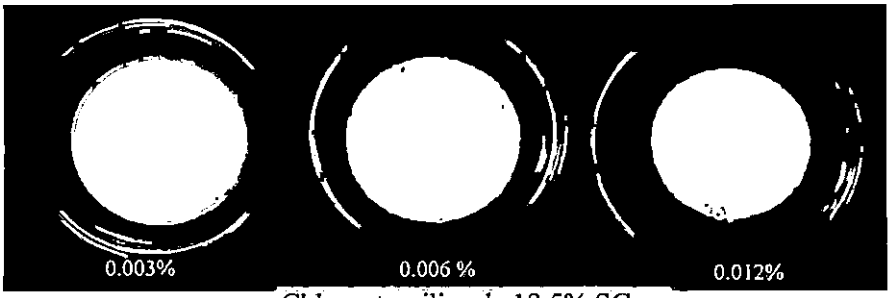
Table 3. Effect of insecticides on growth, sporulation and germination of *Lecanicillium saksenae*

Insecticides	Concentrations (%)	Dose	Radial growth (cm) *		Spore count (10^7 spores mL^{-1})*	Germination (%) **
			7 DAI	14 DAI	21 DAI	
Flubendiamide 39.35% SC	0.0025	0.5x	3.30 (1.82)^{b}	7.47 (2.73)^{b}	4.31 (2.06)^{abc}	92.33 (74.07)^{cde}
	0.005	1x	3.07(1.75)^{cde}	7.40 (2.72)^{bc}	4.09 (2.00)^{abc}	89.00 (71.04)^{ef}
	0.01	2x	2.97 (1.72)^{f}	7.37(2.71)^{bc}	3.46 (1.85)^{bc}	89.00 (70.83)^{ef}
Chlorantraniliprole 18.5% SC	0.003	0.5x	3.23 (1.8)^{bcd}	7.13(2.67)^{cde}	4.68 (2.16)^{ab}	91.67 (73.77) ^{de}
	0.006	1x	3.17 (1.78)^{bcd}	6.83(2.61)^{efg}	4.16 (2.01)^{abc}	90.00 (71.62)^{e}
	0.012	2x	3.00 (1.73)^{ef}	6.63 (2.58)^{fg}	3.83 (1.93)^{abc}	84.33 (66.83)^{fg}
Imidacloprid 17.8% SL	0.003	0.5x	3.27 (1.81)^{bc}	7.27 (2.7)^{bcd}	4.72 (2.17)^{ab}	95.33 (77.64)^{bcd}
	0.006	1x	3.20(1.79)^{bcde}	7.03 (2.65)^{de}	4.23 (2.06)^{abc}	93.00 (74.68)^{cde}
	0.012	2x	3.03 (1.74)^{def}	6.93 (2.63)^{ef}	3.58 (1.89)^{abc}	88.33 (70.45)^{ef}
Thiamethoxam 25% WG	0.0025	0.5x	3.23 (1.8)^{bcd}	7.40 (2.72)^{bc}	4.46 (2.11)^{abc}	97.00 (80.12)^{b}
	0.005	1x	3.17 (1.78)^{bcd}	7.13(2.67)^{cde}	4.04 (1.99)^{abc}	95.67 (78.49)^{bc}
	0.01	2x	3.07 (1.75)^{cde}	6.60 (2.57)^{g}	4.13 (2.03)^{abc}	92.00 (73.64)^{de}
Malathion 50% EC	0.05	0.5x	1.50 (1.22)^{i}	4.53 (2.13)^{i}	0.30 (0.55)^{ef}	79.33 (63.02)^{ghi}
	0.1	1x	1.43 (1.2)^{i}	3.50 (1.87)^{k}	0.28 (0.53)^{ef}	77.00 (61.35)^{hij}
	0.2	2x	1.4 (1.18)^{ij}	3.40 (1.84)^{k}	0.23 (0.47)^{efg}	71.00 (57.43)^{j}
Quinalphos 25% EC	0.025	0.5x	1.27 (1.13)^{j}	2.60 (1.61)^{m}	0.06 (0.25)^{fg}	63.33 (52.74)^{k}
	0.05	1x	1.10 (1.05)^{k}	2.47 (1.57)^{m}	0.07 (0.25)^{fg}	52.33 (46.34)^{lm}
	0.1	2x	1.03 (1.02)^{k}	1.60 (1.26)^{n}	0.03 (0.18)^{g}	44.67 (41.93)^{m}
Dimethoate 30% EC	0.02	0.5x	2.43 (1.56)^{g}	7.43(2.73)^{bc}	4.11 (2.03)^{abc}	82.67 (65.42)^{gh}
	0.04	1x	2.30 (1.52)^{g}	7.40(2.72)^{bc}	3.29 (1.79)^{e}	80.67 (64.04)^{gh}
	0.08	2x	2.03 (1.43)^{h}	5.10 (2.26)^{h}	0.30 (0.54)^{ef}	78.67 (62.52)^{ghi}
Chlorpyrifos 20% EC	0.03	0.5x	2.27 (1.51)^{g}	3.83 (1.96)^{j}	0.87 (0.93)^{d}	73.00 (58.71)^{ij}
	0.06	1x	2.00 (1.41)^{h}	3.33 (1.83)^{k}	0.51 (0.71)^{de}	62.67 (52.34)^{k}
	0.12	2x	1.97 (1.4)^{h}	3.10 (1.76)^{l}	0.34 (0.58)^{ef}	56.33 (48.64)^{kl}
Control			4.33 (2.08)^{a}	7.90 (2.81)^{a}	4.86 (2.21)^{a}	100 (89.71)^{a}
CD (0.05)			(0.064)	(0.061)	(0.34)	(4.663)
±S.Em			0.10	0.28	0.39	3.00

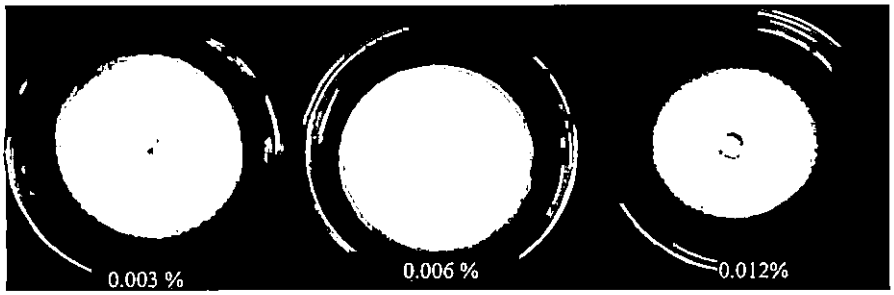
1x- recommended dose, DAI-Days After Inoculation, * Values in parentheses are square root transformed, ** Values in parentheses are arc sine transformed.



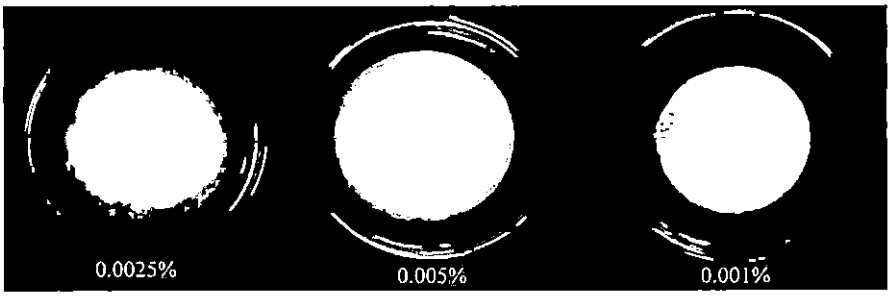
Flubendiamide 39.35% EC



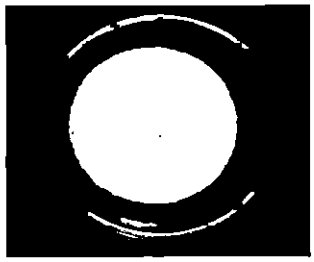
Chlorantraniliprole 18.5% SC



Imidacloprid 17.8% SL

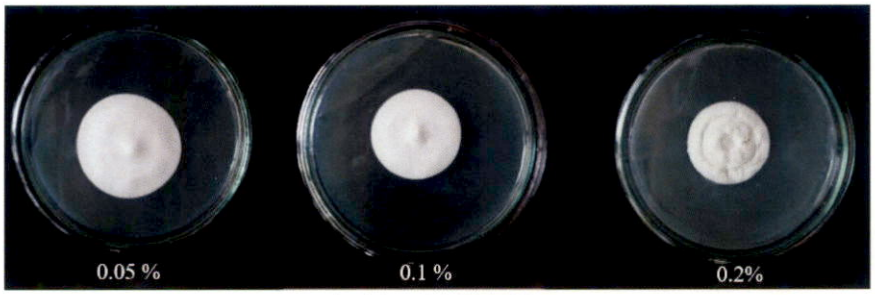


Thiamethoxam 25% WG



Control

Plate 1. Colony growth of *Lecanicillium saksenae* on media poisoned with new generation insecticides at 14 days after inoculation

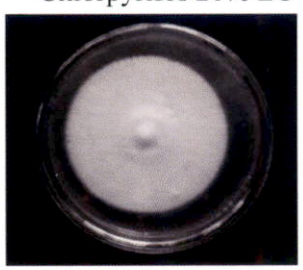


Malathion 50 % EC

Quinalphos 25% EC

Dimethoate 30% EC

Chlorpyrifos 20% EC



Control

Plate 2. Colony growth of *Lecanicillium saksenae* on media poisoned with old generation insecticides at 14 days after inoculation

Among the old generation insecticides media poisoned with dimethoate 30% EC 0.02 % and chlorpyriphos 20% EC 0.03 % displayed significantly higher radial growth of 2.43 cm and 2.27 cm, respectively. Malathion 50% EC 0.05 % exhibited 1.50 cm growth, while least growth was observed in quinalphos 25% EC 0.025% (1.27 cm). The corresponding growth observed in unpoisoned medium was 4.33 cm.

On the 14th DAI, unpoisoned medium exhibited radial growth of 7.90 cm, while flubendiamide 39.35% SC 0.0025 % exhibited 7.47 cm which was statistically on par with that of dimethoate 30% EC 0.02 % (7.43 cm), thiamethoxam 25% WG 0.0025 % (7.40 cm) and imidacloprid 17.8% SL 0.003 % (7.27 cm). Medium poisoned with chlorantraniliprole 18.5% SC 0.003 % exhibited growth of 7.13 cm. Among the old generation insecticides, malathion 50% EC 0.005 % exhibited 4.53 cm radial growth, which varied significantly from that of chlorpyriphos 20% EC 0.03 % (3.83 cm) and quinalphos 25% EC 0.025 % (2.60 cm).

4.1.1.1.2 At Recommended Dose

Analysis of data recorded on the seventh DAI revealed that, *L. saksenae* is highly tolerant to all four new generation insecticides. Among these, media poisoned with imidacloprid 17.8% SL 0.006 % exhibited highest colony diameter (3.20 cm) which was statistically on par with chlorantraniliprole 18.5% SC 0.006% and thiamethoxam 25% WG 0.005 % with no growth variation between them (3.17 cm). Growth in flubendiamide 39.35% SC 0.005 % was 3.07 cm. Among the old generation insecticides, the medium poisoned with dimethoate 30% EC 0.04 % displayed significantly more radial growth (2.30 cm) compared to chlorpyriphos 20% EC 0.06 % (2.00 cm) and malathion 50 EC 0.1 % with no variation among them (1.43 cm). Least growth was noted in quinalphos 25% EC 0.05 % (1.10 cm).

On the 14th DAI growth in dimethoate 30% EC 0.04 % and flubendiamide 39.35% SC 0.005 % were on par (7.40 cm). Growth in thiamethoxam 25% WG

0.005 %, imidacloprid 17.8% SL 0.006 % and chlorantraniliprole 18.5 % SC 0.006 % did not vary significantly with mean radial growth of 7.13 cm, 7.03 cm and 6.83 cm respectively, but was significantly lower than the former. Malathion 50% EC 0.1 % exhibited 3.50 cm growth, while in chlorpyriphos 20% EC 0.06 % it was 3.33 cm which were statistically similar to each other. Least growth was noted in quinalphos 25% EC 0.05 % (2.47 cm).

4.1.1.1.3 At Double the Recommended Dose

On the seventh DAI, among the poisoned media thiamethoxam 25% WG 0.01 % exhibited highest growth (3.07 cm) which was statistically on par with growth in imidacloprid 17.8% SL 0.012 % (3.03 cm), chlorantraniliprole 18.5% SC 0.012 % (3.00 cm). and flubendiamide 39.35% SC 0.01 % (2.97 cm). Growth in dimethoate 30% EC 0.08 % and chlorpyriphos 20% EC 0.12 % did not vary significantly, with mean radial growth of 2.03 cm and 1.97 cm respectively. Medium with malathion 50% EC 0.2 % recorded a growth of 1.40 cm and the least growth was noted in quinalphos 25% EC 0.1 % (1.03 cm).

On the 14th DAI, flubendiamide 39.35% SC 0.01 % exhibited highest growth (7.37 cm) which was lower than that observed in the unpoisoned medium (7.90 cm). Mycelial growth in imidacloprid 17.8% SL 0.012 % (6.93 cm) and chlorantraniliprole 18.5% SC 0.012 % (6.63 cm) were statistically similar followed by that in thiamethoxam 25% WG 0.01 % (6.60 cm). Among the old generation insecticides, dimethoate 30% EC 0.08 % displayed significantly high radial growth (5.10 cm) compared to malathion 50% EC 0.2 % (3.40 cm) and chlorpyrifos 20% EC 0.12 % (3.10 cm). Least growth was observed in quinalphos 25% EC 0.01 % (1.60 cm).

4.1.1.2 Sporulation

4.1.1.2.1 At Half the Recommended Dose

Data on sporulation of *L. saksenae* recorded on the 21st day in poisoned media revealed that, the new generation insecticides were more compatible

compared to the old ones. The spore load was 10^7 spores mL^{-1} in all new generation insecticides while it was drastically reduced in media poisoned with old generation insecticides.

Analysis of data revealed that the medium poisoned with imidacloprid 17.8% SL 0.003 % and chlorantraniliprole 18.5% SC 0.003 % recorded the highest spore count of 4.72 x 10^7 spores mL^{-1} and 4.68 x 10^7 spores mL^{-1} respectively, which were statistically on par with those in the unpoisoned media (4.86 x 10^7 spores mL^{-1}). Spore count in thiamethoxam 25% WG 0.0025 % and flubendiamide 39.35 % SC 0.0025 % was 4.46 x 10^7 spores mL^{-1} and 4.31 x 10^7 spores mL^{-1}, respectively which were statistically on par with each other. Among the old generation insecticides, highest spore count of 4.11 x 10^7 spores mL^{-1} was observed in dimethoate 30% EC 0.02%. There was an exponential reduction in sporulation when the medium was poisoned with chlorpyrifos 20% EC 0.03 % (8.7 x 10^6 spores mL^{-1}) and malathion 50% EC 0.05 % (3.0 x 10^6 spores mL^{-1}). Least sporulation (6.0 x 10^5 spores mL^{-1}) was recorded in quinalphos 25% EC 0.025 %.

4.1.1.2.2 At Recommended Dose

There was no significant variation in the spore count among imidacloprid 17.8% SL 0.006 % (4.23 x 10^7 spores mL^{-1}), chlorantraniliprole 18.5% SC 0.006 % (4.16 x 10^7 spores mL^{-1}) flubendiamide 39.35% SC 0.005 % (4.09 x 10^7 spores mL^{-1}) and thiamethoxam 25% WG 0.005 % (4.04 x 10^7 spores mL^{-1}). The count in old generation insecticide dimethoate 30% EC 0.04 % ranked second (3.29 x 10^7 mL^{-1}). Sporulation in chlorpyrifos 20% EC 0.06 % and malathion 50% EC 0.1 % was drastically lowered to 10^6, but did not vary significantly vary from each other. It was 5.1 x 10^6 spores $mL^{-1}$and 2.8 x 10^6 spores mL^{-1} respectively. It was quinalphos 25% EC 0.05 % that highly affected the sporulation of *L. saksenae* (7.0 x 10^5 spores mL^{-1}).

4.1.1.2.3 At Double the Recommended Dose

Sporulation of *L. saksenae* was highest in the medium poisoned with thiamethoxam 25% WG 0.01 % (4.13 x 10^7 mL^{-1}) followed by those in chlorantraniliprole 18.5% SC 0.012 % (3.83 x 10^7 mL^{-1}), imidacloprid 17.8% SL 0.012 (3.58 x 10^7 mL^{-1}) and flubendiamide 39.35% SC 0.01 % (3.46 x 10^7 mL^{-1}). All the old generation insecticides, significantly reduced the spore load to 10^6 or below. Least spore count (3.0 x 10^5 spores mL^{-1}) was recorded in quinalphos 25% EC 0.1 %.

4.1.1.3 Germination

Conidial germination, 24 h after exposure to insecticides is expressed in percentage.

4.1.1.3.1 At Half the Recommended Dose

Germination rate of *L. saksenae* after 24 h exposure to insecticides revealed that it was affected by all the insecticides tested. Germination was comparatively more in new generation insecticides. Highest germination (97.00 per cent) was recorded in thiamethoxam 25% WG 0.0025 %, followed by imidacloprid 17.8% SL 0.003 % (95.33 per cent), flubendiamide 39.35% SC 0.0025 % (92.33 per cent) and chlorantraniliprole 18.5% SC 0.003 % (91.67 per cent), which were on par with each other. Germination percentage for dimethoate 30% EC 0.02 % was lower (82.67 per cent). The treatments malathion 50% EC 0.05 % (79.33 per cent) and chlorpyrifos 20% EC 0.03 % (73.00 per cent) significantly affected the germination of spores. Quinalphos 25% EC 0.025 % exhibited the least germination of 63.33 per cent. Corresponding germination observed in unpoisoned medium was 100 per cent.

4.1.1.3.2 At Recommended Dose

Highest germination rate of 95.67 per cent was observed in thiamethoxam 25% WG 0.005 %, while imidacloprid 17.8% SL 0.006 % ranked second (93.00

per cent). In chlorantraniliprole 18.5% SC 0.006 % and flubendiamide 39.35% SC 0.005 % the rate was 90.00 and 89.00 per cent, respectively, which were on par with each other. Among the old generation insecticides, dimethoate 30% EC 0.04 % exhibited highest germination (80.67 per cent) which was statistically on par with malathion 50% EC 0.1 % (77.00 per cent). Chlorpyrifos 20% EC 0.06 % and quinalphos 25% EC 0.05 % significantly reduced the rate of germination (62.67 and 52.33 per cent, respectively).

4.1.1.3.3 At Double the Recommended Dose

Highest germination rate of 92.00 per cent was noted in thiamethoxam 25% WG 0.01 %, followed by flubendiamide 39.35% SC 0.01 % and imidacloprid 17.8% SL 0.012 % with 89.00 and 88.33 per cent germination, respectively. The rate of germination was significantly low in chlorantraniliprole 18.5% SC 0.012 % and dimethoate 30% SC 0.08 % (84.33 and 78.67 per cent respectively), which were statistically on par with each other. The germination capacity was significantly lowered in malathion 50% EC 0.2 % (71 per cent) and chlorpyrifos 20% EC 0.12 % (56.33 per cent). Least germination (44.67 per cent) was observed in quinalphos 25% EC 0.1 %. In untreated control the radial growth was 100 per cent.

4.1.2 *L. lecanii*

The growth parameters of *L. lecanii* in insecticide poisoned media were presented in Table 4.

4.1.2.1 Radial Growth

Radial growth of the fungus in terms of mean colony diameter, recorded on tenth and 21st DAI in PDA poisoned with insecticides is presented in Plates 3 and 4.

4.1.2.1.1 At Half the Recommended Dose

Analysis of data recorded on the tenth DAI revealed that, *L. lecanii* is highly tolerant to the new generation insecticide, imidacloprid 17.8% SL 0.003 %

Flubendiamide 39.35 % EC

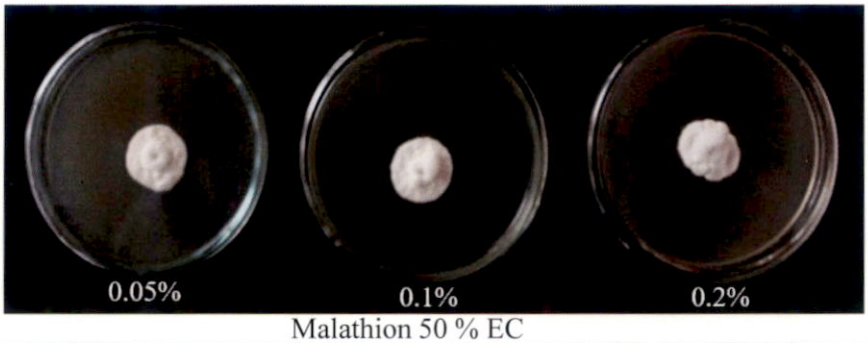
Chlorantraniliprole 18.5 % SC

Imidacloprid 17.8 % SL

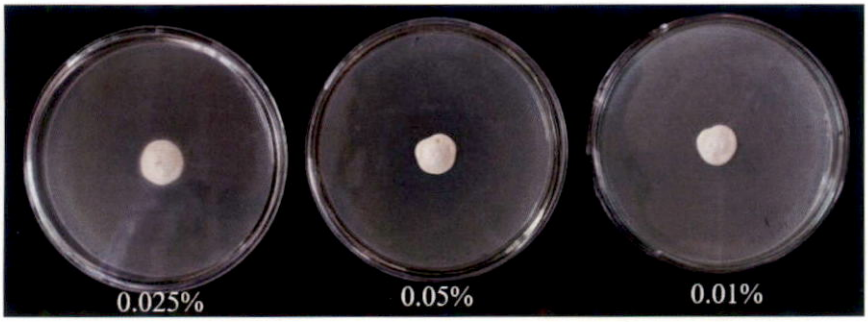
Thiamethoxam 25 % WG

Control

Plate 3. Colony growth of *Lecanicillium lecanii* on media poisoned with new generation insecticides at 21 DAI

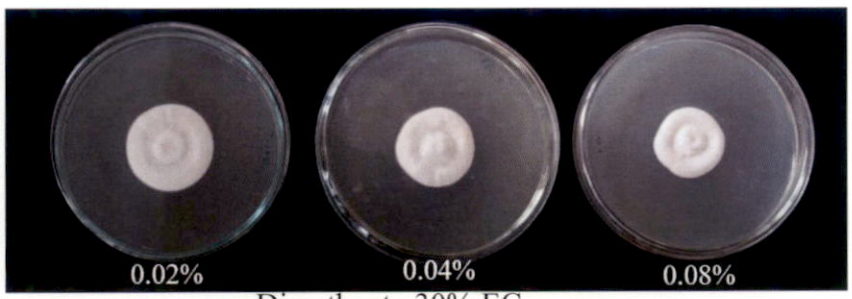


Malathion 50 % EC



Quinalphos 25% EC

Chlorpyrifos 20% EC



Dimethoate 30% EC

Control

Plate 4. Colony growth of *Lecanicillium lecanii* on media poisoned with old generation insecticides at 21 DAI

wherein the mean colony diameter was 2.33 cm. The mycelial growth in flubendiamide 39.35% SC 0.0025 %, thiamethoxam 25% WG 0.0025 % and chlorantraniliprole 18.5% SC 0.003 % did not vary significantly, with mean radial growth of 2.23 cm, 2.13 cm and 2.10 cm respectively.

Among the old generation insecticides, the medium poisoned with dimethoate 30% EC 0.02 % displayed significantly more radial growth (1.73 cm) compared to chlorpyriphos 20% EC 0.03 % (1.53 cm), followed by malathion 50% EC 0.05 % and quinalphos 25% EC 0.025 % (1.40 cm and 0.80 cm respectively) The growth observed in unpoisoned medium was 2.47 cm.

On the 21st DAI, imidacloprid 17.8% SL 0.003 % and thiamethoxam 25% WG 0.0025 % exhibited a similar rate of growth with no variation among them (4.40 cm) followed by chlorantraniliprole 18.5% SC 0.003 % and flubendiamide 39.35% SC 0.0025 % (4.20 cm and 3.90 cm respectively). The medium poisoned with dimethoate 30% EC 0.02 % displayed significantly more radial growth (3.63 cm) compared to other old generation insecticides such as chlorpyriphos 20% EC 0.03 % (2.70 cm) and malathion 50% EC 0.05 % (2.47 cm) which varied significantly from that in quinalphos 25% EC 0.025 % (1.10 cm). Unpoisoned medium exhibited radial growth of 4.47 cm, which was significantly higher than all the other treatments. Medium without insecticides exhibited 4.47 cm growth, which was significantly higher than all the other treatments.

4.1.2.1.2 At Recommended Dose

On the 10th DAI, among the poisoned media thiamethoxam 25% WG 0.005 % and imidacloprid 17.8% SL 0.006 % exhibited highest growth, 2.20 cm and 2.17 cm respectively while in untreated control it was 2.47 cm which was significantly higher than all other treatment. Growth in flubendiamide 39.35% SC 0.005 % and chlorantraniliprole 18.5% SC 0.006 % did not vary significantly, with mean radial growth of 2.13 cm and 2.07 cm respectively.

Table 4. Effect of insecticides on growth, sporulation and germination of *Lecanicillium lecanii*

Insecticides	Concentrations (%)	Dose	Radial growth (cm) *		Spore count (10^7 spores mL^{-1}) *	Germination (%) **
			7 DAI	14 DAI	21 DAI	
Flubendiamide 39.35% SC	0.0025	0.5x	2.23 (1.49)^{bc}	3.90 (1.98)^{d}	2.96 (1.72)^{cd}	93.00 (74.74)^{b}
	0.005	1x	2.13 (1.46)^{c}	3.70 (1.92)^{e}	2.48 (1.57)^{cde}	92.67 (74.34)^{bc}
	0.01	2x	2.10 (1.45)^{c}	3.40 (1.84)^{f}	2.36 (1.54)^{def}	87.33(69.36)^{defghi}
Chlorantraniliprole 18.5% SC	0.003	0.5x	2.10 (1.45)^{c}	4.20(2.05)^{cd}	3.09 (1.76)^{bc}	89.67(71.25)^{cdefg}
	0.006	1x	2.07 (1.44)^{c}	4.10(2.03)^{bc}	2.27 (1.51)^{ef}	87.00 (68.99)^{efghi}
	0.012	2x	1.87 (1.37)^{d}	3.90 (1.98)^{d}	1.98 (1.41)^{ef}	85.33 (67.59)^{hi}
Imidacloprid 17.8% SL	0.003	0.5x	2.33 (1.53)^{ab}	4.40(2.10)^{ab}	3.01 (1.72)^{bcd}	90.33 (71.92)^{bcde}
	0.006	1x	2.17 (1.47)^{bc}	4.30(2.07)^{abc}	2.21 (1.49)^{ef}	86.33 (68.36)^{fghi}
	0.012	2x	2.13 (1.46)^{c}	4.10 (2.03)^{cd}	1.80 (1.34)^{fg}	85.00 (67.32)^{i}
Thiamethoxam 25% WG	0.0025	0.5x	2.13 (1.46)^{c}	4.40 (2.10)^{ab}	3.73 (1.93)^{ab}	91.00 (72.56)^{bcd}
	0.005	1x	2.20 (1.48)^{bc}	4.20 (2.05)^{bc}	2.46 (1.55)^{cde}	89.67 (71.38)^{cdef}
	0.01	2x	2.13 (1.46)^{c}	4.10 (2.02)^{cd}	2.45 (1.56)^{cde}	86.00 (68.05)^{ghi}
Malathion 50% EC	0.05	0.5x	1.40 (1.18)^{gh}	2.47 (1.57)^{i}	0.38 (0.59)^{hi}	74.67 (59.82)^{j}
	0.1	1x	1.47 (1.21)^{g}	2.30 (1.52)^{j}	0.31 (0.53)^{i}	71.67 (57.89)^{jk}
	0.2	2x	1.30 (1.14)^{h}	2.20 (1.48)^{j}	0.05 (0.22)^{j}	70.33 (57.02)^{jk}
Quinalphos 25% EC	0.025	0.5x	0.80 (0.89)^{i}	1.10 (1.05)^{k}	0.06 (0.25)^{j}	52.33 (46.34)^{mn}
	0.05	1x	0.80 (0.89)^{i}	1.10 (1.05)^{k}	0.04 (0.18)^{j}	47.00 (43.28)^{n}
	0.1	2x	0.77 (0.87)^{i}	1.00 (1.00)^{k}	0.03 (0.16)^{j}	32.67 (34.85)^{op}
Dimethoate 30% EC	0.02	0.5x	1.73 (1.32)^{de}	3.63 (1.91)^{e}	2.18 (1.48)^{ef}	90.33 (71.92)^{bcde}
	0.04	1x	1.67 (1.29)^{ef}	3.60 (1.90)^{e}	2.00 (1.41)^{ef}	89.00(70.64)^{defgh}
	0.08	2x	1.50 (1.22)^{g}	2.90 (1.7)^{g}	1.34 (1.16)^{g}	87.67(69.46)^{defghi}
Chlorpyrifos 20% EC	0.03	0.5x	1.53 (1.24)^{fg}	2.70 (1.64)^{h}	0.58 (0.76)^{h}	68.33 (55.76)^{kl}
	0.06	1x	1.47 (1.21)^{g}	2.50 (1.58)^{i}	0.48 (0.69)^{hi}	63.33 (52.74)^{l}
	0.12	2x	1.27 (1.13)^{h}	2.27 (1.51)^{j}	0.36 (0.6)^{hi}	53.33 (46.91)^{m}
Control			2.47 (1.57)^{a}	4.47 (2.11)^{a}	4.11 (2.03)^{a}	100 (89.71)^{a}
CD (0.05)			(0.063)	(0.051)	(0.207)	(3.244)
±S.Em			0.069	0.107	0.251	3.411

1x- Recommended dose, DAI - Days After Inoculation, * Values in parentheses are square root transformed ** Values in parentheses are arc sine transformed.

Among the old generation insecticides, the medium poisoned with dimethoate 30% EC 0.04 % displayed significantly more radial growth (1.67 cm) compared to chlorpyriphos 20% EC 0.06 % and malathion 50% EC 0.1 % with no variation among them (1.47 cm). Least growth was noted in quinalphos 25% EC 0.05 % (0.8 cm).

On the 21st DAI, imidacloprid 17.8% SL 0.006 % exhibited highest radial growth (4.30 cm) followed by thiamethoxam 25% WG 0.005 % (4.20 cm) and chlorantraniliprole 18.5% SC 0.006 % (4.10 cm). Mycelial growth in flubendiamide 39.35% SC 0.005 % (3.70) and dimethoate 30% EC 0.04 % (3.60 cm) was statistically similar. Chlorpyriphos 20% EC 0.06 % exhibited growth of 2.50 cm which was statistically different from malathion 50% EC 0.1 % (2.30 cm). Quinalphos 25% EC 0.05 % exhibited least growth (1.10 cm). In the control a radial growth of 4.47 cm was noted which was significantly higher than all the other treatments.

4.1.2.1.3 At Double the Recommended Dose

On the 10th DAI, among the poisoned media imidacloprid 17.8% SL 0.012 %, thiamethoxam 25% WG 0.01 % and flubendiamide 39.35% SC 0.01 % exhibited highest growth, 2.13 cm, 2.13 cm and 2.10 cm respectively which were significantly lower than that in unpoisoned medium (2.47 cm). Mycelial growth in chlorantraniliprole 18.5% SC 0.012 % was 1.87 cm. Among the old generation insecticides, the medium poisoned with dimethoate 30% EC 0.08 % displayed significantly more radial growth (1.50 cm) compared to malathion 50% EC 0.2% (1.30 cm) and chlorpyriphos 20% EC 0.12 % (1.27 cm). Least growth was noted in quinalphos 25% EC 0.1 % (0.77 cm).

On the 21st DAI, imidacloprid 17.8% SL 0.012 % and thiamethoxam 25% WG 0.01 % exhibited highest radial growth with no variation among them (4.10 cm) followed by chlorantraniliprole 18.5% SC 0.012 % (3.90 cm). Mycelial growth in flubendiamide 39.35% SC 0.01 % (3.40) and dimethoate 30% EC

0.05 % (2.90 cm) varied significantly among themselves. Chlorpyriphos 20% EC 0.12 % exhibited growth of 2.27 cm which was statistically different from malathion 50% EC 0.2 % (2.20 cm). Quinalphos 25% EC 0.1 % exhibited least growth (1.00 cm). In the control plate there was a radial growth of 4.47 cm, which was significantly higher than all the other treatments.

4.1.2.2 Sporulation

4.1.2.2.1 At Half the Recommended Dose

Data on sporulation of *L. lecanii* recorded on the 21st day, in poisoned medium revealed that, new generation insecticides were more compatible compared to the old ones. The spore load was 10^7 spores mL^{-1}in all new generation insecticides while it was drastically reduced in media poisoned with old generation insecticides.

Analysis of data revealed that among the treatments highest sporulation (3.73×10^7 mL^{-1}) was there in medium poisoned with thiamethoxam 25% WG 0.0025 % which was statistically lower than that observed in unpoisoned medium (4.11×10^7 mL^{-1}). Spore count in chlorantraniliprole 18.5% SC 0.003 % and imidacloprid 17.8% SL 0.003 % was 3.09×10^7 spores $mL^{-1}$and 3.01×10^7 spores mL^{-1}respectively, which did not vary significantly among each other. Sporulation in flubendiamide 39.35% SC 0.0025 % was 2.96×10^7 mL^{-1}. Among the old generation insecticides, highest sporulation (2.18×10^7 mL^{-1}) was observed in dimethoate 30% EC 0.025 %. There was an exponential reduction in media poisoned with chlorpyrifos 20% EC 0.03 % (5.8×10^6 mL^{-1}) and malathion 50% EC 0.05 % (3.8×10^6 mL^{-1}). Least sporulation (6.0×10^5 mL^{-1}) was recorded in quinalphos 25% EC 0.025 %.

4.1.2.2.2 At Recommended Dose

Among the treatments the sporulation was highest in media poisoned with flubendiamide 39.35% SC 0.005 % (2.48×10^7 mL^{-1}) and thiamethoxam 25% WG 0.005 % (2.46×10^7 mL^{-1}) followed by those in chlorantraniliprole 18.5% SC

0.006 % (2.27 x 10^7 mL^{-1}), imidacloprid 17.8% SL 0.006 % (2.21 x 10^7 mL^{-1}) and dimethoate 30% EC 0.04 % (2.00 x 10^7 mL^{-1}). Sporulation in chlorpyrifos 20% EC 0.06 % and malathion 50% EC 0.1 % was drastically lowered to 10^6 spores mL^{-1} but did not vary significantly vary from each other. It was 4.80 x 10^6 spores mL^{-1} and 3.10 x 10^6 mL^{-1} respectively. It was quinalphos 25% EC 0.05% that highly affected the sporulation (4.00 x 10^5 mL^{-1}). The corresponding sporulation in control was 4.11 x 10^7 spores mL^{-1}.

4.1.2.2.2 At Double the Recommended Dose

Sporulation of *L. lecanii* was highest in the medium poisoned with thiamethoxam 25% WG 0.01% (2.45 x 10^7 mL^{-1}) followed by those in flubendiamide 39.35% SC 0.01% (2.36 x 10^7 mL^{-1}), chlorantraniliprole 18.5% SC 0.012% (1.98 x 10^7 mL^{-1}) and imidacloprid 17.8% SL 0.012% (1.80 x 10^7 mL^{-1}). Among old generation insecticides, media with dimethoate 30% EC 0.08% exhibited highest sporulation (1.34 x 10^7 mL^{-1}). Chlorpyrifos 20% EC 0.12% and malathion 50% EC 0.2% significantly reduced the spore load to 10^6 (3.6 x 10^6 mL^{-1}) and 10^5 (5.00 x10^5 mL^{-1}). Least spore count (3 x 10^5 mL^{-1}) was recorded in quinalphos 25% EC 0.1% and the highest was in untreated control 4.11 x 10^7 spores mL^{-1}.

4.1.2.3 Germination

4.1.2.3.1 At Half the Recommended Dose

Germination of *L. lecanii* was affected by all the insecticides tested. Germination percentage was comparatively more in new generation insecticides. Flubendiamide 39.35% SC 0.0025 % recorded 93 per cent, thiamethoxam 25% WG 0.0025 % 91 per cent and imidacloprid 17.8% SL 0.003 % 90.33 per cent, which were on par. The germination percentage was 90.33 per cent in dimethoate 30% EC 0.02 % which was equal to that calculated in imidacloprid 17.8% SL 0.003 %. Germination percentage calculated for chlorantraniliprole 18.5% SC 0.003 % was lower (89.67 per cent). The treatments malathion 50% EC 0.05 % (74.67 per cent) and chlorpyrifos 20% EC 0.03 % (68.33 per cent) significantly

affected the germination of spores. Quinalphos 25% EC 0.0025 % exhibited the least germination of 52.33 per cent. Corresponding germination observed in unpoisoned medium was 100 per cent.

4.1.2.3.2 At Recommended Dose

Among the treatments highest germination (92.67 per cent) was observed in flubendiamide 39.35% SC 0.005 %. Thiamethoxam 25% WG 0.005 % and dimethoate 30% EC 0.04 % ranked second (89.67 per cent and 89.00 per cent respectively), which were on par with each other. Chlorantraniliprole 18.5% SC 0.006 % (87 per cent) and imidacloprid 17.8% SL 0.006 % (86.33 per cent) also affected germination. Among old generation insecticides malathion 50% EC 0.1 % and chlorpyrifos 20% EC 0.06 % significantly affected germination where germination of 71.67 per cent and 63.33 per cent was recorded. Least germination (47.00 per cent) was noticed in quinalphos 25% EC 0.05 %. In unpoisoned media 100 per cent germination was observed.

4.1.2.3.3 At Double the Recommended Dose

Highest germination noted among the treatments was in dimethoate 30% EC 0.08 % and flubendiamide 39.35% SC 0.01 %, which were on par with each other. Germination percentage observed was 87.67 and 87.33, respectively. This was followed by thiamethoxam 25% WG 0.01 % (86.00 per cent), chlorantraniliprole 18.5% SC 0.012 % (85.33 per cent) and imidacloprid 17.8% SL 0.012 % (85.00 per cent) which were significantly different from each other. Malathion 50% EC 0.2 % (70.33 per cent) and chlorpyrifos 20% EC 0.12 % (53.33 per cent) significantly affected the spore germination. Least germination (32.67 per cent) was observed in quinalphos 25% EC 0.1 %. In unpoisoned media 100 per cent germination was observed.

4.1.3 Compatibility Status

Compatibility status of *L. saksenae and L. lecanii* with different chemical insecticides is indicated in Table 5.

Table 5. Compatibility status of *Lecanicillium saksenae* and *Lecanicillium lecanii* with insecticides

Insecticides	Concentrations (%)	Dose	*L. saksenae*		*L. lecanii*	
			BI	Compatibility status	BI	Compatibility status
Flubendiamide 39.35% SC	0.0025	0.5x	92	Compatible	82	Compatible
	0.005	1x	89	Compatible	75	Compatible
	0.01	2x	83	Compatible	70	Compatible
Chlorantraniliprole 18.5% SC	0.003	0.5x	93	Compatible	85	Compatible
	0.006	1x	86	Compatible	77	Compatible
	0.012	2x	82	Compatible	71	Compatible
Imidacloprid 17.8% SL	0.003	0.5x	95	Compatible	88	Compatible
	0.006	1x	89	Compatible	78	Compatible
	0.012	2x	82	Compatible	71	Compatible
Thiamethoxam 25% WG	0.0025	0.5x	93	Compatible	95	Compatible
	0.005	1x	88	Compatible	80	Compatible
	0.01	2x	85	Compatible	78	Compatible
Malathion 50% EC	0.05	0.5x	38	Toxic	38	Toxic
	0.1	1x	31	Toxic	35	Toxic
	0.2	2x	29	Toxic	31	Toxic
Quinalphos 25% EC	0.025	0.5x	22	Toxic	18	Toxic
	0.05	1x	21	Toxic	17	Toxic
	0.01	2x	14	Toxic	14	Toxic
Dimethoate 30% EC	0.02	0.5x	89	Compatible	70	Compatible
	0.04	1x	81	Compatible	69	Compatible
	0.08	2x	41	Toxic	54	Moderately Toxic
Chlorpyrifos 20% EC	0.03	0.5x	38	Toxic	42	Toxic
	0.06	1x	31	Toxic	38	Toxic
	0.12	2x	27	Toxic	33	Toxic

1x- recommended dose; BI-Biological index, 0 to 41 = toxic; 42 to 66 = moderately toxic; >66 = compatible.

All the new generation insecticides were found to be compatible based on their BI, both at half, recommended and double the recommended doses in both the fungi. In *L. saksenae,* BI varied from 82 in chlorantraniliprole 18.5% SC 0.012 % to 95 in imidacloprid 17.8% SL 0.003 %. In *L. lecanii* BI index varied from 70 in flubendiamide 39.35% SC 0.01 % to 95 in thiamethoxam 25% WG 0.0025 %.

The old generation insecticide dimethoate 30% EC was compatible at half dose (0.02%) and recommended dose (0.04 %) in both the fungi, whereas at double the recommended dose (0.08 %) it was toxic to *L. saksenae* and moderately toxic to *L. lecanii* (BI value of 41 and 54 respectively). Other old generation insecticides such as malathion 50% EC, quinalphos 25% EC and chlorpyrifos 20% EC were toxic to both the fungi at all the three doses.

4.2 TOLERANCE OF *L. saksenae* AND *L. lecanii* TO FUNGICIDES

Tolerance of *L. saksenae* and *L. lecanii* with old generation fungicides such as copper oxychloride 50% WP, carbendazim 50% WP and mancozeb 75% WP and new generation fungicides such as azoxystrobin 23% SC, hexaconazole 5% EC and tebuconazole 25% EC was assessed in terms of radial growth, sporulation and germination.

4.2.1 *L. saksenae*

As fungi are generally susceptible to fungicides, compatibility was tested at recommended dose (Table 6) and then tolerance studies were carried out with the non-compatible fungicides.

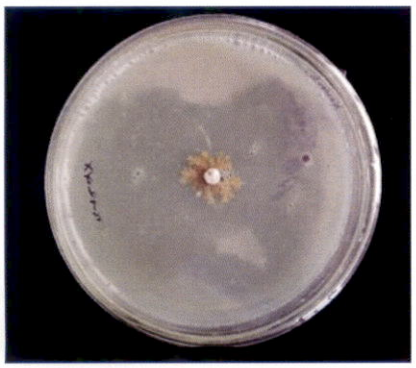
4.2.1.1 Radial Growth

Analysis of data recorded on the seventh DAI and 14th DAI revealed that, *L. saksenae* was highly susceptible to fungicides and its growth was exhibited in only two fungicides (Plate 5). The radial growth observed in azoxystrobin 23% SC 0.1% was 2.9 cm and 8.47 cm on the seventh and 14th DAI respectively.

Table 6. Effect of fungicides on growth, sporulation and germination of *Lecanicillium saksenae*

Fungicides	Concentrations (%)	Radial growth (cm)*		Spore count (10^7 spores mL^{-1})*	Germination (%)**
		7 DAI	14 DAI	21 DAI	
Copper oxychloride 50% WP	0.2	0 (0.71)^{d}	0 (0.71)^{c}	0 (0.71)^{d}	97.67 (82.73)^{a}
Azoxystrobin 23% SC	0.1	2.90 (1.84)^{b}	8.47 (2.99)^{a}	2.33 (1.68)^{b}	95.67 (78.49)^{b}
Carbendazim 50%WP	0.2	0.80 (1.14)^{c}	2.40 (1.7)^{b}	0.03 (0.73)^{c}	28.33 (32.08)^{b}
Mancozeb 75% WP	0.3	0 (0.71)^{d}	0 (0.71)^{c}	0 (0.71)^{d}	0 (0.29)^{c}
Hexaconazole 5% EC	0.15	0 (0.71)^{d}	0 (0.71)^{c}	0 (0.71)^{d}	0 (0.29)^{c}
Tebuconazole 25%EC	0.2	0 (0.71)^{d}	0 (0.71)^{c}	0 (0.71)^{d}	0 (0.29)^{c}
Control		4.33 (2.2)^{a}	8.63 (3.02)^{a}	5.37 (2.41)^{a}	100 (89.71)^{c}
CD (0.05)		(0.029)	(0.059)	(0.234)	(5.525)
S.Em ±		0.666	1.525	0.783	10.262

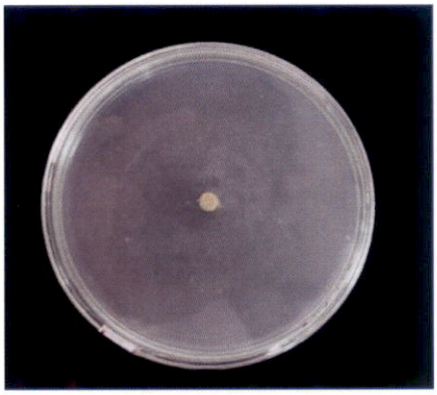
DAI - Days After Inoculation * Values in parentheses are square root transformed values ** Values in parentheses are arc sine transformed.



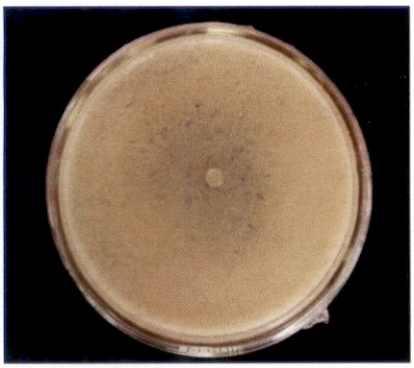
Copper oxychloride 50% WP- 0.2%

Azoxystrobin 23% SC -0.1%

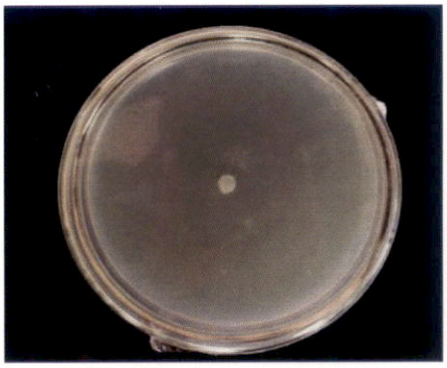
Carbendazim 50% WP- 0.2%



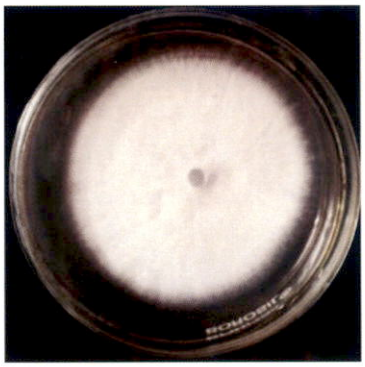
Hexaconazole 5% EC- 0.15%



Mancozeb 75% WP- 0.3%



Tebuconazole 25% EC- 0.2%



Control

Plate 5. Colony growth of *Lecanicillium saksenae* on media poisoned with fungicides at 14 days after inoculation

Growth was observed only in carbendazim 50% WP 0.8 % poisoned medium, where 0.8 cm and 2.4 cm were observed on respective days. There was no mycelial growth in the media poisoned with copper oxychloride 50% WP 0.2 %, mancozeb 75% WP 0.3 %, hexaconazole 5% EC 0.15 % and tebuconazole 25% EC 0.2 %. Growth of *L. saksenae* in unpoisoned medium was 4.33 cm and 8.63 cm on the seventh and 14th DAI respectively, which was significantly higher than that observed in the treatments.

4.2.1.2 Sporulation

Copper oxychloride 50% WP 0.2 %, mancozeb 75% WP 0.3 %, hexaconazole 5% EC 0.15 % and tebuconazole 25% EC 0.2 % completely inhibited sporulation as observed on 21 DAI. Media poisoned with azoxystrobin 23% SC 0.1 % exhibited sporulation of 2.33 x 10^7 spores mL^{-1} which was significantly lower from unpoisoned medium (5.37 x 10^7 mL^{-1}). In carbendazim 50% WP poisoned medium, exponential reduction in sporulation was observed (3.0 x10^5 mL^{-1}).

4.2.1.3 Germination

Spore germination of 97.67 per cent and 95.67 per cent was observed in media poisoned with copper oxychloride 50% WP 0.2 % and azoxystrobin 23% SC 0.1 % respectively, which were statistically on par with each other. Medium poisoned with carbendazim 50% WP 0.2 % exhibited 28.33 per cent germination, while in the untreated medium 100 per cent germination was observed. None of spore germinated in media poisoned with mancozeb 75% WP 0.3 %, hexaconazole 5% EC 0.15 % and tebuconazole 25% EC 0.2 %.

4.2.2 *L. lecanii*

The growth parameters of *L. lecanii* grown in media poisoned with fungicides is summarised in Table 7.

Table 7. Effect of fungicides on growth, sporulation and germination of *L. lecanii*

Fungicides	Concentrations (%)	Radial growth (cm)*		Spore count ($10^7 mL^{-1}$)*	Germination (%)**
		10 DAI	21DAI	21 DAI	
Copper oxychloride 50% WP	0.2	1.50 (1.41)^{b}	3.27 (1.94)^{b}	0.37 (0.93)^{c}	98.33 (83.87)^{ab}
Azoxystrobin 23% SC	0.1	1.33 (1.35)^{c}	2.97 (1.86)^{c}	0.60 (1.05)^{b}	95.67 (78.49)^{b}
Carbendazim 50%WP	0.2	0.00 (0.71)^{d}	0.00 (0.71)^{e}	0.00 (0.71)	0 (0.29)^{c}
Mancozeb 75% WP	0.3	1.27 (1.33)^{c}	2.40 (1.7)^{d}	0.04 (0.73)^{d}	0 (0.29)^{c}
Hexaconazole 5% EC	0.15	0 (0.71)^{d}	0 (0.71)^{e}	0 (0.71)	0 (0.29)^{c}
Tebuconazole 25%EC	0.2	0 (0.71)^{d}	0 (0.71)^{e}	0 (0.71)	0 (0.29)^{c}
Control		2.67 (1.78)^{a}	5.10 (2.37)^{a}	4.19 (2.17)^{a}	99.00 (86.48)^{a}
CD (0.05)		(0.054)	(0.063)	(0.066)	(5.790)
S.Em ±		0.384	0.761	0.581	19.735

DAI - Days after inoculation * Values in parentheses are square root transformed
** Values in parentheses are arc sine transformed.

4.2.2.1 Radial Growth

Mean colony diameter of *L. lecanii* in PDA poisoned with different fungicides is presented in Plate 6.

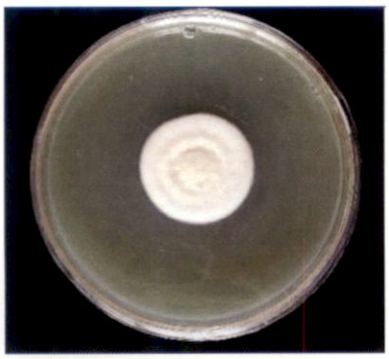
Analysis of data recorded on the tenth and 21st DAI, revealed that, *L. lecanii* was highly susceptible to fungicides. Highest growth was observed in medium poisoned with copper oxychloride 50% WP 0.2 % with mean colony diameter of 1.5 cm and 3.27 cm on the tenth and 21st DAI respectively. This was significantly more than the growth observed in azoxystrobin 23% SC 0.1 % with 1.33 cm and 2.97 cm. Medium poisoned with mancozeb 75% WP 0.3 % exhibited 1.27 and 2.40 cm colony diameter on the tenth and 21st DAI respectively. Corresponding growth observed in the untreated medium was 2.67 cm and 5.10 cm. No mycelial growth was observed in media poisoned with carbendazim 50% WP 0.2 %, hexaconazole 5% EC 0.15 % and tebuconazole 25% EC 0.2 %.

4.2.2.2 Sporulation

Spore count of *L. saksenae* on poisoned media with fungicides was assessed 21 DAI. Carbendazim 50% WP 0.2 %, hexaconazole 5% EC 0.15 % and tebuconazole 25%EC 0.2 % completely inhibited the sporulation. Those in which there was sporulation, the count was exponentially reduced. In azoxystrobin 23% SC 0.1 % the spore count was 6.0×10^6 spores mL^{-1} which was significantly lower from control (4.19×10^7 mL^{-1}). Spore count in copper oxychloride 50% WP 0.2 % and mancozeb 75% WP 0.3 % was 3.7×10^6 spores mL^{-1} and 4.0×10^5 spores mL^{-1} respectively, which varied significantly from each other statistically.

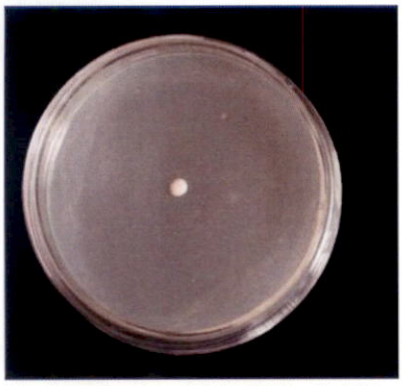
4.2.2.3 Germination

A high germination rate of 98.33 per cent and 95.67 per cent was observed in media poisoned with copper oxychloride 50% WP 0.2 % and azoxystrobin 23% SC 0.1 % respectively which were statistically on par with each other, while in untreated spores the rate was 99 per cent. None of spore germinated in media poisoned with carbendazim 50%WP 0.2 %, mancozeb 75% WP 0.3 %, hexaconazole 5% EC 0.15 % and tebuconazole 25% EC 0.2 %.

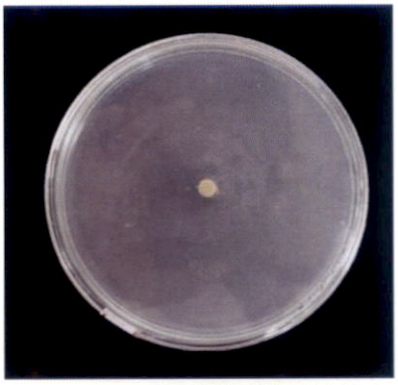


Copper oxychloride 50% WP- 0.2%

Azoxystrobin 23% SC- 0.1%



Carbendazim 50% WP- 0.2%



Hexaconazole 5% EC- 0.15%

Mancozeb 75% WP- 0.3%

Tebuconazole 25% EC- 0.2%

Control

Plate 6. Colony growth of *Lecanicillium lecanii* on media poisoned with fungicides at 21 days after inoculation

4.2.3 Compatibility Status

Compatibility status of *L. saksenae and L. lecanii* with fungicides based on BI index is indicated in Table 8.

Azoxystrobin 23% SC 0.1 % was compatible with *L. saksenae* and moderately toxic to *L. lecanii*. Copper oxychloride 50% WP 0.2 % was toxic to *L. saksenae*, whereas it was moderately toxic to *L. lecanii*. Carbendazim 50% WP 0.2%, mancozeb 75% WP 0.3 %, hexaconazole 5% EC 0.15 % and tebuconazole 25% EC 0.2 % were toxic to both the fungi.

As *L. saksenae* was compatible with azoxystrobin 23% SC 0.1 %, other fungicides were tested for tolerance. *L. lecanii* was not compatible with any of the tested fungicides and hence its tolerance was tested with all the six fungicides.

4.3 ARTIFICIAL SELECTION FOR INSECTICIDE TOLERANCE

As a pre requisite for artificial selection, Highest Tolerant Dose (HTD) of incompatible insecticides was determined based on their growth parameters in poisoned media. The doses tested were those selected based on results mentioned in Para 4.1 and 4.2.

4.3.1 Highest Tolerant Dose

L. saksenae and *L. lecanii* was incompatible with malathion 50% EC, quinalphos 25% EC, and chlorpyriphos 20% EC. The most toxic insecticide quinalphos 25% EC was selected to test the HTD and to induce tolerance. HTD of both the fungi to quinalphos 25% EC is indicated in Table 9.

4.3.1.1 L. saksenae

Tolerance was assessed at 0.05 %, 0.1 % and 0.2 %. Media poisoned with quinalphos 25% EC 0.05 % exhibited colony growth, germination and sporulation.

Table 8. Compatibility status of *Lecanicillium saksenae* and *Lecanicillium lecanii* with fungicides at recommended dose

Fungicides	Concentrations (%)	*L. saksenae*		*L. lecanii*	
		BI	Compatibility status	BI	Compatibility status
Copper oxychloride 50% WP	0.2	10	Toxic	44.00	Moderately toxic
Azoxystrobin 23% SC	0.1	74	Compatible	46.00	Moderately toxic
Carbendazim 50%WP	0.2	16	Toxic	0	Toxic
Mancozeb 75% WP	0.3	0	Toxic	23.00	Toxic
Hexaconazole 5% EC	0.15	0	Toxic	0	Toxic
Tebuconazole 25%EC	0.2	0	Toxic	0	Toxic

BI- Biological index, 0 to 41 = toxic; 42 to 66 = moderately toxic; >66 = compatible.

Table 9. Highest Tolerance Dose of *Lecanicillium saksenae* and *Lecanicillium lecanii* to quinalphos 25 % EC

Fungi	Concentrations (%)	Radial growth ± S.E (cm)	Spore count ± S.E (10^7 spores mL^{-1})	Germination ± S.E (%)
L.saksenae	0.05	2.47 ± 0.033	0.07 ± 0.019	52.33 ± 1.73
	0.1*	1.60 ± 0.058	0.03 ± 0.008	44.67 ± 2.12
	0.2	0	0	0
L. lecanii	0.05	1.10 ± 0.06	0.04 ± 0.01	47.00 ± 1.16
	0.1*	1.00 ± 0.06	0.03 ± 0.01	32.67 ± 0.89
	0.2	0.00	0.00	0.00

S.E - Standard Error, * Highest Tolerant Dose, DAI - Days After Inoculation

Colony growth of 2.47 cm and 1.60 cm, sporulation of 7 x 10^5 spores mL^{-1} and 3 x 10^5 spores mL^{-1} and germination of 52.33 per cent and 44.67 per cent was observed at 0.05% and 0.1% respectively. At 0.2%, all the three growth parameters were totally arrested. Therefore, 0.1% was determined as HTD for quinalphos 25% EC.

4.3.1.2 L. lecanii

Tolerance was assessed at 0.05 %, 0.1 % and 0.2 % concentrations. Media poisoned with quinalphos 25% EC 0.05 % and 0.1 % exhibited colony growth, germination and sporulation and at 0.2 % concentration all the three-growth parameters terminated. Colony growth of 1.1 cm and 1.0 cm, sporulation of 4 x 10^5 spores mL^{-1} and 3 x 10^5 spores mL^{-1} and germination of 47.00 per cent and 32.67 per cent was observed at 0.05 % and 0.1 % respectively. Therefore, 0.1 % was determined as HTD.

4.3.2 Induced Tolerance to Quinalphos 25% EC

Growth parameters of *L. saksenae* and *L. lecanii* induced for tolerance to quinalphos 25% EC is presented in Table 10.

4.3.2.1 L. saksenae

Quinalphos tolerance induced in *L. saksenae* did not result in significant change of phenotypic characters over 10 generation. Colony diameter varied between 1.40 cm (highest value observed in the third generation) and 1.11 cm (lowest in the eighth generation) and sporulation from 5.9 x 10^5 spores mL^{-1} in tenth generation to 2.48 x 10^5 spores mL^{-1} in the first. Germination rate varied between 42.34 per cent in the fourth generation to 19.67 per cent in the eighth.

4.3.2.2 L. lecanii

Quinalphos tolerance induced in *L. lecanii* was also found to have no changes in phenotypic characters over 10 successive generations.

Table 10. Growth parameters of *Lecanicillium saksenae* and *Lecanicillium lecanii* induced for quinalphos 25 % EC tolerance.

Generation	*L. saksenae*			*L. lecanii*		
	Radial growth (cm)* 14 DAI	Spore count (10^5 spores mL^{-1}) * 14 DAI	Germination (%)**	Colony growth (cm)* 14 DAI	Spore count (10^5 spores mL^{-1})* 14 DAI	Germination (%)**
I	1.37 (1.17)	2.48 (1.53)	32.34 (34.54)	1.13 (1.06)	4.81 (2.03)	34.33 (35.79)
II	1.34 (1.16)	4.4 (1.97)	34.00 (35.44)	1.20 (1.1)	4.07 (1.87)	44.00 (41.55)
III	1.40 (1.19)	5.21 (2.24)	35.67 (36.63)	1.40 (1.18)	5.54 (2.32)	37.33 (37.66)
IV	1.28 (1.13)	5.34 (2.24)	42.34 (40.59)	1.37 (1.17)	6.13 (2.31)	35.67 (36.63)
V	1.34 (1.16)	4.81 (2.14)	30.34 (33.29)	1.53 (1.24)	5.14 (2.22)	41.00 (39.75)
VI	1.28 (1.13)	4.77 (2.05)	30.67 (33.53)	1.50 (1.22)	5.43 (2.28)	33.00 (35.06)
VII	1.32 (1.15)	4.44 (2.05)	31.34 (33.99)	1.67 (1.29)	4.77 (2.15)	34.33 (35.44)
VIII	1.11 (1.06)	4.9 (2.15)	19.67 (26.21)	1.47 (1.21)	5.57 (2.33)	45.67 (42.49)
IX	1.17 (1.08)	5.40 (2.19)	27.6 (31.66)	1.40 (1.18)	6.73 (2.58)	37.00 (37.15)
X	1.24 (1.11)	5.90 (2.33)	26.34 (30.83)	1.37 (1.17)	5.23 (2.2)	26.33 (30.83)
CD (0.05)	NS	NS	NS	NS	NS	NS
S.Em±	0.28	2.9	1.89	0.049	0.23	1.78

DAI-Days After Inoculation, NS- Non-Significant, * Values in parentheses are square root transformed ** Values in parentheses are arc sine transformed

Colony growth varied between 1.67 cm (highest value observed in the seventh generation) and 1.13 cm (lowest in the first); sporulation from 6.73 x 10^5 spores mL^{-1} in the ninth to 4.07 x 10^5 spores mL^{-1} in the second generation. Rate of germination varied between 44.00 per cent in the second to 26.33 per cent in the eighth.

4.3.3 Performance of Insecticide Tolerant strains

Comparative tolerance of the "selected" cultures (sub cultured with incompatible insecticide for 10 successive generations at HTD), with those of "relaxed" culture (sub cultured for seven successive generations in poisoned medium and the next three generations in the unpoisoned medium) and the non-selected" culture (normal culture) is depicted below.

Comparative tolerance of *L. saksenae* and *L. lecanii* to quinalphos 25% EC, assessed at recommended dose at 0.05 % is presented in Table 11.

4.3.3.1 L. saksenae

No significant difference was observed between the selected, relaxed and non-selected cultures in terms of growth, sporulation and germination.

The radial growth was 2.03, 1.78 and 2.13 cm respectively in selected, relaxed and non-selected cultures, while the sporulation was 1.45 x 10^5 spores mL^{-1}, 1.53 x 10^5 spores mL^{-1} and 2.55 x 10^5 spores mL^{-1} respectively. The germination percentage ranged from 45.83 to 49.33.

4.3.3.2 L. lecanii

No significant difference was noted between the three types. Radial growth, was 1.24, 1.31 and 1.30 cm respectively in selected, relaxed and non-selected cultures sporulation was 4.48 x 10^5 spores mL^{-1}, 4.2 x10^5 spores mL^{-1} and 5.33 x10^5 spores mL^{-1} and germination percentage noted was 35.83. 34.50 and 42.00, respectively

Table 11. Comparative growth parameters of selected, relaxed and non-selected cultures in quinalphos 25 % EC poisoned medium at recommended dose (0.01 %)

Culture	*L. saksenae*			*L. lecanii*		
	Radial growth (cm)* 14 DAI	Sporulation (10^5 spores mL^{-1}) * 21 DAI	Germination (%)**	Radial growth (cm)* 21 DAI	Sporulation (10^5 spores mL^{-1}) * 21 DAI	Germination (%)**
Selected	2.03 (1.42)	1.45 (1.17)	45.83 (42.61)	1.24 (1.11)	4.48 (1.96)	35.80 (36.71)
Relaxed	1.78 (1.33)	1.53 (1.19)	46.17 (42.8)	1.31 (1.14)	4.20 (1.73)	34.5 (35.89)
Non selected	2.13 (1.46)	2.55 (1.54)	49.33 (44.62)	1.30 (1.14)	5.33 (2.27)	42.00 (40.38)
CD (0.05)	NS	NS	NS	NS	NS	NS
S.Em ±	0.47	0.211	1.122	0.12	0.44	1.52

NS- Non-significant; DAI-Days after inoculation. * Values in parentheses are square root transformed values ** Values in parentheses are arc sine transformed.

No improvement in growth parameters was observed in the insecticide tolerance induced strains compared to the relaxed or non-selected cultures of both the spp. Hence, no genotypic evaluation was conducted.

4.4 ARTFICIAL SELECTION FOR FUNGICIDE TOLERANCE

4.4.1 Highest Tolerant Dose

4.4.1.1 L. saksenae

L. saksenae was found to be incompatible with carbendazim 50% WP, hexaconazole 5% EC, copper oxychloride 50% WP, mancozeb 75% WP and tebuconazole 25% EC. The HTD value of these fungicide are depicted in Table 12.

4.4.1.1 1 Carbendazim 50% WP

Tolerance was assessed at 0.2 %, 1% and 1.2% concentrations. Colony growth, germination and sporulation observed in carbendazim 50% WP 0.2% and 1% was 2.4 cm and 1.73 cm; 3×10^5 mL^{-1} and 1×10^5 spores mL^{-1} and 81.33 per cent and 5.33 per cent at 0.05% and 0.1% respectively. As all the growth parameters terminated at 1.2 %, 1% was determined as HTD.

4.4.1.1.2 Hexaconazole 5% EC

Tolerance was assessed at 0.015 %, 0.03 % and 0.06 % concentrations. The colony growth, germination and sporulation were observed only at 0.015 %. Colony growth of 2.73 cm, sporulation of 1×10^5 spores mL^{-1} and germination of 56.67 per cent was observed at this concentration. No growth and sporulation were observed at higher concentrations. Thus, 0.015 % was determined as HTD.

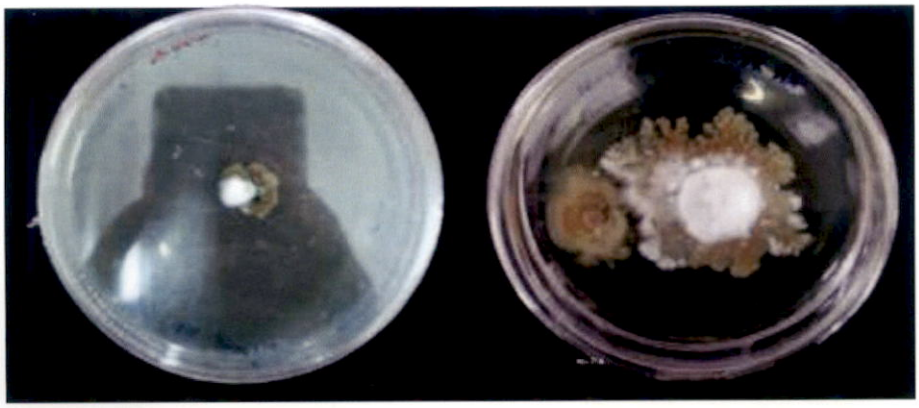
4.4.1.1.3 Copper oxychloride 50% WP

Tolerance was tested at $1/10^{th}$ of recommended dose i.e., 0.02 %. No growth and sporulation were observed at 14 DAI. Germination percentage of 99.33 per cent was recorded at this dose. However different type of growth was observed in some petri plates as depicted in Plate 7.

Table 12. Highest tolerant dose of *Lecanicillium saksenae* to incompatible fungicides

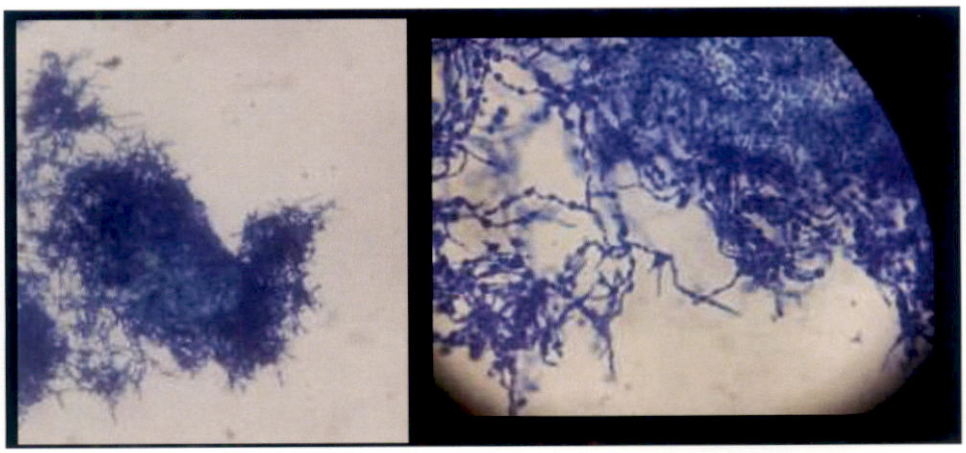
Fungicide	Concentrations (%)	Dose	Radial growth (cm) ± S.E	Spore count (10^7 spores mL^{-1}) ± S.E	Germination (%) ± S.E
			14 DAI	21DAI	
Carbendazim 50%WP	0.20	1x	2.40 ± 0.06	0.03 ± 0.012	28.33 ±3.283
	1*	5x	1.73 ± 0.12	0.01 ± 0.025	5.33 ± 0.33
	1.20	6x	0	0	0
Hexaconazole 5% EC	0.015*	0.1x	2.73 ± 0.07	0.01 ± 0.0017	56.67 ± 6.67
	0.03	0.2x	0	0	0
	0.06	0.3x	0	0	0
Copper oxychloride 50% WP	0.02	0.1x	0	0	99.33 ± 0.67
Mancozeb 75% WP	0.03	0.1x	0	0	0
Tebuconazole 25%EC	0.02	0.1x	0	0	0
Control	0.02	-	8.63	5.37	100

S.E - Standard Error, DAI - Days after inoculation, 1x- recommended dose, *Highest Tolerant Dose



0.2% 0.1%

Control



10x magnification 40 x magnification

Plate 7. Morphological changes in *Lecanicillium saksenae* on copper oxychloride 50 % WP poisoned medium at 30 days after inoculation

4.4.1.1.4 Mancozeb 75% WP

Tolerance was tested at $1/10^{th}$ of recommended dose *i.e.* 0.03 %. No growth, germination and sporulation were observed at this dose. Hence, no further attempt was made to induce tolerance.

4.4.1.1.5 Tebuconazole 25% EC

At $1/10^{th}$ recommended dose *i.e.,* 0.02% tebuconazole 25% EC completely inhibited the growth, sporulation and germination. Hence, no further attempt was made to induce tolerance.

4.4.1.2 L. lecanii

L. lecanii was incompatible with all the tested fungicides. In copper oxychloride 50% WP and azoxystrobin 23% SC minimum dose tested was recommended dose as it exhibited growth (but incompatible) at this dose in compatibility studies (para 4.2.3). HTD value is indicated in Table 13.

4.4.1.2.1 Copper oxychloride 50% WP

Tolerance was assessed at 0.2 %, 1 % and 1.2 % concentration. The colony growth, germination and sporulation were observed in copper oxychloride 50% WP 0.2 % and 1 %. It was 3.27 cm and 2.14 cm, 3.7×10^6 spores mL^{-1} and 5×10^5 spores mL^{-1} and 98.34 per cent and 9.67 per cent at 0.05 % and 0.1 % respectively. All the growth parameters were found to be arrested at 1.2 %. Thus, 1.00 % concentration was determined as HTD

4.4.1.2.2 Azoxystrobin 23% SC

Tolerance was assessed at 0.1 %, 0.5 % and 0.6 % concentration. Colony growth of 3.27 cm, sporulation of 2.3×10^6 spores mL^{-1} and germination of 95.67 per cent was observed at 0.1% and at 0.5 % the corresponding values were 3.07 cm, 5.3×10^5 spores mL^{-1} and 7.67 per cent respectively. All the growth parameters terminated at 0.6 %. Therefore, 0.5 % was determined as HTD.

4.4.1.2.3 Mancozeb 75% WP

Tolerance was assessed at 0.03 %, 0.15 %, 0.30 % and 0.60 % concentrations. Colony growth of 2.7 cm, 2.63 cm, 2.4 cm and sporulation of 5.6×10^6 mL^{-1}, 3.4×10^6 mL^{-1}, 4.0×10^5 spores mL^{-1} was recorded at 0.03 %, 0.15 % and 0.30 % concentrations respectively. No germination was recorded at all the four tested concentrations. Therefore, 0.3 % was set as HTD to induce tolerance.

4.4.1.2.4 Carbendazim 50% WP

Tolerance was tested at $1/10^{th}$ of recommended dose i.e. 0.02 %, no growth, germination and sporulation were observed at this dose. Hence, no further attempt was made to induce tolerance.

4.4.1.2.5 Hexaconazole 5% EC

Tolerance was tested at $1/10^{th}$ the recommended dose i.e. 0.015 %. No growth, germination and sporulation were observed at this dose. Hence, no further attempt was made to induce tolerance.

4.4.1.2.6 Tebuconazole 25% EC

Tolerance was tested at $1/10^{th}$ of recommended dose i.e. 0.02 % and was found to be totally arrested and hence it was not induced for tolerance.

4.4.2 Induced Tolerance to Fungicides

4.4.2.1 L. saksenae

Growth characters of *L. saksenae* over 10 successive generations when grown in HTD of carbendazim 50% WP and hexaconazole 5% EC is depicted in Table 14.

4.4.2.1.1 Carbendazim 50% WP

There was no significant difference in colony growth during the first four generations, where the mean colony diameter ranged 2.0 to 2.24 cm.

Table 14. Growth parameters of *Lecanicillium saksenae* induced for carbendazim 50% WP tolerance

Generation	Radial growth (cm)* 14 DAI	Spore count (10^5spores ml^{-1}) * 14 DAI	Germination (%)**
I	2.07 (1.44)^{c}	0.49 (0.7)^{cd}	6.00 (14.15)^{c}
II	2.00 (1.42)^{c}	0.54 (0.7)^{cd}	5.34 (13.27)^{c}
III	2.24 (1.50)^{c}	0.56 (0.72)^{cd}	5.67 (13.46)^{c}
IV	2.20 (1.49)^{c}	0.33 (0.55)^{d}	8.67 (16.89)^{bc}
V	2.74 (1.66)^{bc}	0.58 (0.76)^{cd}	14.34 (22.22)^{a}
VI	3.40 (1.85)^{ab}	1.24 (1.11)^{bc}	13.67 (21.66)^{a}
VII	3.97 (2.00)^{a}	2.7 (1.63)^{a}	13.00 (21.13)^{ab}
VIII	3.27 (1.80)^{ab}	2.90 (1.69)^{a}	11.67 (19.9)^{ab}
IX	3.40 (1.84)^{ab}	1.87 (1.33)^{ab}	12.67 (20.65)^{ab}
X	3.27 (1.80)^{ab}	1.50 (1.21)^{b}	12.34 (20.46)^{ab}
CD (0.05)	(0.258)	(0.419)	(4.450)
S.Em ±	1.124	0.302	1.124

DAI - Days After Inoculation, * Values in parentheses are square root transformed , ** Values in parentheses are arc sine transformed

In the fifth and sixth generations there was significant increase in growth, the mean diameter being 2.74 cm and 3.40 cm respectively. Highest growth of 3.97 cm was observed in seventh generation which was statistically on par with that observed in next three generations (3.27 cm, 3.4 cm and 3.27 cm in the eighth, ninth and 10^{th} respectively).

No significant difference in sporulation was observed in the first five generations with lowest sporulation of 3.3×10^4 spores mL^{-1} recorded in the fourth and the highest of 5.8×10^4 spores mL^{-1} in the fifth. Logarithmic increase in sporulation was observed from the sixth generation onwards.

It was 1.24×10^5 spores mL^{-1} in the sixth, 2.7×10^5 spores mL^{-1} in the seventh and 2.9×10^5 spores mL^{-1} in the eighth, which were on par with each other. Sporulation in the ninth and tenth generations differed significantly from each other (1.87×10^5 spores mL^{-1} and 1.50×10^5 spores mL^{-1}).

Conidial germination did not vary significantly during the first four generations (5.34 per cent to 8.67 per cent). Thereafter it increased to 14.34 per cent in the fifth and 13.67 per cent in the sixth which were on par. In the subsequent generations the percentage did not vary significantly (11.67 to 13.00).

4.4.2.1.2 Hexaconazole 5% EC

Phenotypic characters of *L. saksenae* induced for hexaconazole tolerance, assessed in terms of growth parameters are presented in Table 15.

Colony growth recorded was 2.47 cm and 2.44 cm during first and second generations respectively. When the concentration of chemical was increased to 0.045%, the third generation exhibited a growth of 2.04 cm diameter, while in the 4^{th} generation it was further increased to 2.7 cm. At a concentration of 0.075% *L. saksenae* exhibited growth of 2.3 and 1.94 cm respectively during fifth and sixth generations.

Table 15. Growth parameters of *Lecanicillium saksenae* induced for hexaconazole 5% SC tolerance

Generation	Concentrations (%)	Colony growth (cm)* 14 DAI	Spore count (10^5spores mL^{-1})* 14 DAI	Germination (%)**
I	0.015	2.47 (1.57)	4.34 (2.06)	45.67 (42.47)^{ab}
II	0.015	2.44 (1.56)	4.33 (2.02)	49.34 (44.63)^{a}
III	0.045	2.04 (1.43)	3.00 (1.71)	13.67 (21.68)^{d}
IV	0.045	2.70 (1.63)	9.00 (2.87)	16.67 (24.04)^{d}
V	0.075	2.30 (1.52)	6.00 (2.42)	11.34 (19.62)^{d}
VI	0.075	1.94 (1.39)	4.07 (1.96)	11.00 (19.28)^{d}
VII	0.12	1.74 (1.31)	4.00 (1.96)	14.34 (22.22)^{d}
VIII	0.12	1.87 (1.36)	6.84 (2.56)	28.67 (32.32)^{c}
IX	0.15	1.86 (1.36)	5.90 (2.4)	32.67 (34.82)^{c}
X	0.15	1.79 (1.34)	7.5 (2.74)	36.34 (37.04)^{bc}
CD (0.05)		NS	NS	5.38
± S.Em		0.106	0.50	4.60

DAI-Days after inoculation. * Values in parentheses are square root transformed values, ** Values in parentheses are arc sine transformed

Thereafter the mycelial growth did not decrease significantly even though the concentration was increased to 0.12 %, the colony diameter noted was 1.74 cm and 1.87 cm, respectively. On further increase to 0.15 % (recommended dose) in the ninth generation the growth was 1.86 cm and at the same concentration in tenth generation it was 1.79 cm in diameter.

Analysis of data on spore count revealed that there was no significant difference in sporulation of *L. saksenae* when the concentration of hexaconazole 5 % EC was increased from 0.0015 % in the 1st generation to 0.15 % in the tenth. It was observed that its tolerance increased by 10 times over the 10 successive generations. At 0.015 % the spore load was 4.34 x 10^5 spores mL^{-1} and 4.33 x 10^5 spores mL^{-1} during the first two generations respectively. No significant reduction in sporulation (3.00 x 10^5 spores mL^{-1}) was observed with increase in concentration to 0.045 % (3rd generation).

The spore count during the 4th generation (9.00 x 10^5 spores mL^{-1}) was on par with that of the fifth (6.00 x 10^5 spores mL^{-1}) . At a concentration of 0.075 %, the spore load was 6.00 x 10^5 mL^{-1} and 4.07 x 10^5 spores mL^{-1} in the fifth and sixth generations respectively. At 0.12 %, the seventh and eighth generations exhibited a spore load of 4.00 x 10^5 spores mL^{-1} and 6.84 x 10^5 spores mL^{-1} respectively. At 0.15 %, the recommended dose, ninth and tenth generations had a spore load of 5.90 x 10^5 spores mL^{-1} and 7.5 x 10^5 spores mL^{-1}, respectively.

There was significant variation in the rate of germination of spores, over the 10 generations. Highest germination of 49.34 per cent was recorded in the 2nd (0.015 % concentration) followed by 45.67 per cent in the 1st generation. At 0.045 % concentration germination significantly reduced to 13.67 per cent and 16.67 per cent in the 3rd and 4th generations respectively. Germination observed in the fifth, sixth and seventh generations was 11.34 per cent, 11.00 per cent and 14.34 per cent respectively, which did not vary among themselves. Germination rate increased significantly in the eighth generation (28.67 per cent) with the concentration of 0.12 %. The corresponding values with the ninth and tenth

generations were 32.67 per cent and 36.34 per cent respectively (at the recommended dose).

4.4.2.2 L. lecanii

Phenotypic characters of *L. lecanii* over 10 successive generations when grown in HTD of the inhibitory fungicides mancozeb 75% WP, copper oxychloride 50% WP and azoxystrobin 23% SC is presented below.

4.4.2.2.1 Mancozeb 75% WP

L. lecanii was able to grow for six generations on mancozeb 75% WP 0.3 % poisoned medium, while its growth was found to be terminated by the seventh generation (Table 16). Colony growth did not vary significantly in the first two generations (mean colony diameter 1.57 cm and 1.93 cm) In the 3rd and 4th generations there was increase in growth (2.17 cm) which was on par with that of the 4th (1.5 cm). Mean colony diameter was 1.33 cm in the fifth generation and thereafter the growth decreased significantly in the sixth generation (0.73 cm). There was no mycelial growth in the seventh generation.

The spore load recorded from the 1st generation was 5.98 x 10^5 mL^{-1} which was significantly higher than all other subsequent generations. It was 4.77 in the 2nd which increased significantly in the 3rd (7.06 x 10^5 spores mL^{-1}) and 4th (5.12 x 10^5 spores mL^{-1}). In the fifth generation spore load was 2.54 x 10^5 mL^{-1} which was significantly higher than that recorded in the sixth generation (4.8 x 10^4 spores mL^{-1}). There was no growth in the seventh generation.

Conidia from all the six generations grown in the medium poisoned with mancozeb 75% WP failed to germinate.

4.4.2.2.2 Azoxystrobin 23% SC

Growth parameters of *L. lecanii* sub cultured for 10 successive generations in medium poisoned with azoxystrobin 23% SC at HTD of 0.5 % did not show any significant decrease or increase in phenotypic characters (Table 17).

Table 16. Growth parameters of *Lecanicillium lecanii* induced for mancozeb 75% WP tolerance

Generation	Colony growth (cm) 14 DAI	Spore count (10^5 spores mL^{-1}) 14 DAI
I	1.57 (1.44)^{ab}	5.98 (2.53)^{a}
II	1.93 (1.56)^{ab}	4.77 (2.29)^{ab}
III	2.17 (1.63)^{a}	7.06 (2.72)^{a}
IV	1.50 (1.41)^{ab}	5.12 (2.35)^{ab}
V	1.33 (1.35)^{bc}	2.54 (1.66)^{bc}
VI	0.73 (1.08)^{c}	0.48 (0.97)^{c}
VII	0 (0.71)^{d}	0 (0.71) ^{d}
CD (0.05)	(0.278)	(0.724)
± S.Em	0.203	0.980

DAI-Days after inoculation; Figures in the parenthesis are square root transformed.

Table 17. Growth parameters of *Lecanicillium lecanii* induced for tolerance to azoxystrobin 23% SC and copper oxychloride 50 % WP

Generation	Azoxystrobin 23% SC 0.5 %			Copper oxychloride 50% WP 1 %		
	Radial growth (cm)* 14 DAI	Spore count (10^5 spores mL^{-1}) * 14 DAI	Germination (%)**	Radial growth (cm)* 14 DAI	Spore count (10^5 spores mL^{-1})* 14 DAI	Germination (%)**
I	2.43 (1.56)	5.63 (2.37)	10.33 (18.69)	1.97 (1.4)	6.90 $(2.6)^{a}$	10.67 (18.98)
II	2.37 (1.54)	3.83 (1.87)	9.00 (17.3)	1.93 (1.39)	5.33 $(2.2)^{ab}$	11.33 (19.48)
III	2.50 (1.58)	3.85 (1.85)	13.67 (21.68)	2.20 (1.48)	5.50 $(2.26)^{ab}$	16.33 (23.69)
IV	2.34 (1.53)	4.60 (2.07)	16.67 (24.04)	1.87 (1.36)	3.53 $(1.86)^{abc}$	15.67 (23.12)
V	2.40 (1.55)	5.27 (2.24)	15.33 (22.97)	2.00 (1.41)	5.13 $(2.21)^{ab}$	16.33 (23.78)
VI	2.50 (1.59)	8.01 (2.83)	14.00 (21.69)	1.93 (1.38)	4.30 $(1.99)^{abc}$	18.67 (25.57)
VII	2.77 (1.66)	7.41 (2.71)	14.33 (22.21)	1.73 (1.32)	1.50 $(1.10)^{cd}$	19.33 (25.93)
VIII	2.48 (1.58)	6.83 (2.55)	10.67 (18.95)	1.80 (1.34)	0.47 $(0.67)^{d}$	17.33 (24.46)
IX	2.50 (1.57)	5.83 (2.34)	11.67 (19.9)	1.82 (1.35)	1.90 $(1.32)^{bcd}$	16.00 (23.44)
X	2.62 (1.62)	6.50 (2.52)	12.00 (20.09)	1.73 (1.32)	1.20 $(1.05)^{cd}$	16.67 (23.97)
CD (0.05)	NS	NS	NS	NS	(0.978)	NS
± S.Em	0.134	0.452	2.910	0.044	0.692	2.20

DAI - Days after inoculation, NS- Non significant, * Values in parentheses are square root transformed ** Values in parentheses are arc sine transformed

Colony growth varied between 2.77 cm (highest value observed in seventh generation) and 2.34 cm (lowest in the 4th generation). In terms of sporulation and germination also there was no significant difference. Sporulation varied from 8.01 x 10^5spores mL^{-1} in the sixth generation to 3.83 x 10^5spores mL^{-1} in the 2nd generation. Germination ranged between 16.67 per cent in 4th generation to 9.0 per cent in the 2nd generation.

4.4.2.2.3 Copper oxychloride 50% WP

L. lecanii when sub cultured for ten successive generations in medium poisoned with copper oxychloride 50% WP at HTD of 1 % did not exhibit any significant change in colony growth and germination across ten generations (Table 17). Colony growth varied between 2.20 cm in third generation to 1.73 cm in seventh generation. Germination varied between 19.33 per cent in seventh generation to 10.67 per cent in first generation.

Significant decrease in sporulation was observed over 10 generations. Highest sporulation (6.90 x 10^5 spores mL^{-1}) was observed in the 1st generation which was statistically on par with the 2nd (5.33 x 10^5 spores mL^{-1}) and 3rd generation (5.50 x 10^5 spores mL^{-1}). Spore load of 3.53 x 10^5 mL^{-1} was recorded in the 4th generation which was statistically on par with the fifth and sixth generations (5.13 x 10^5 spores mL^{-1} and 4.30 x 10^5 spores mL^{-1} respectively). Significant decrease in sporulation was observed in the seventh and eighth generations, with least sporulation of 4.7 x 10^4 spores mL^{-1} in eighth generation. In the ninth and tenth generations sporulation of 1.90 x 10^5 spores mL^{-1} and 1.20 x 10^5 spores mL^{-1} was recorded respectively.

4.4.3 Performance of Fungicide Tolerant strains

4.4.3.1 Phenotypic Character

4.4.3.1.1 L. saksenae

Comparative tolerance between selected, relaxed and non-selected cultures of carbendazim and hexaconazole tolerant strains of *L.saksenae* in terms of growth, sporulation and germination is presented in Table 18.

4.4.3.1.1.1 Carbendazim 50% WP

Colony growth was enhanced significantly (4.12 cm) in the relaxed culture when compared to the selected culture (3.22 cm), while in non-selected culture there was growth inhibition (1.82 cm) (Plate 8). Highest sporulation of 5.98 x 10^5 spores mL^{-1} was recorded in the relaxed culture, while it was 2.79 x 10^5 spores mL^{-1} in the selected culture. There was an exponential reduction in sporulation in non-selected culture (5.8 x 10^4 spores mL^{-1}). Highest germination percentage (96 per cent) was observed in relaxed culture which was par with the selected culture (of 95.17 per cent), while the least was noted (31.33 per cent) in non-selected culture.

Significant increase in growth parameters was observed in relaxed and selected cultures compared to non-selected culture in carbendazim 50% WP poisoned medium thus induction of tolerance was positive.

4.4.3.1.1.2 Hexaconazole 5% EC

Highest radial growth (2.02 cm) was observed in selected culture which was significantly different from relaxed culture (1.63 cm). No growth was observed in non-selected culture (Plate 9). Sporulation of 5.72 x 10^5 spores mL^{-1} was recorded in selected culture which was statistically on par with relaxed culture where sporulation of 4.71 x 10^5 spores mL^{-1} was recorded. No sporulation was observed in non-selected culture. No significant difference in germination was observed between relaxed and selected culture where germination of 37.67 per cent and 33.17 per cent was recorded respectively.

Table 18. Comparative growth parameters of selected, relaxed and non-selected *Lecanicillium saksenae* in fungicide poisoned media at recommended dose

Culture	Carbendazim 50% EC 0.2%			Hexaconazole 5% EC 0.1%		
	Radial growth 21 DAI (cm)*	Spore count (10^5 spores mL^{-1})*	Germination (%)**	Radial growth 21 DAI (cm)*	Spore count (10^5 spores mL^{-1})*	Germination (%)**
Selected	3.22 $(1.79)^b$	2.79 $(1.64)^b$	95.17 $(77.78)^a$	2.02 $(1.59)^a$	5.72 $(2.45)^a$	33.17 $(35.12)^a$
Relaxed	4.12 $(2.03)^a$	5.98 $(2.43)^a$	96.00 $(78.67)^b$	1.63 $(1.46)^b$	4.71 $(2.24)^a$	37.67 $(37.81)^a$
Non selected	1.82 $(1.34)^c$	0.58 $(0.75)^c$	31.33 $(33.97)^c$	0 $(0.71)^c$	0 $(0.71)^b$	0 $(0.29)^b$
CD (0.05)	(0.139)	(0.346)	(4.142)	(0.082)	(0.483)	(3.250)
±S.Em	0.364	0.873	21.418	0.387	1.044	7.11

DAI-Days after inoculation, * Values in parentheses are square root transformed values ** Values in parentheses are arc sine transformed

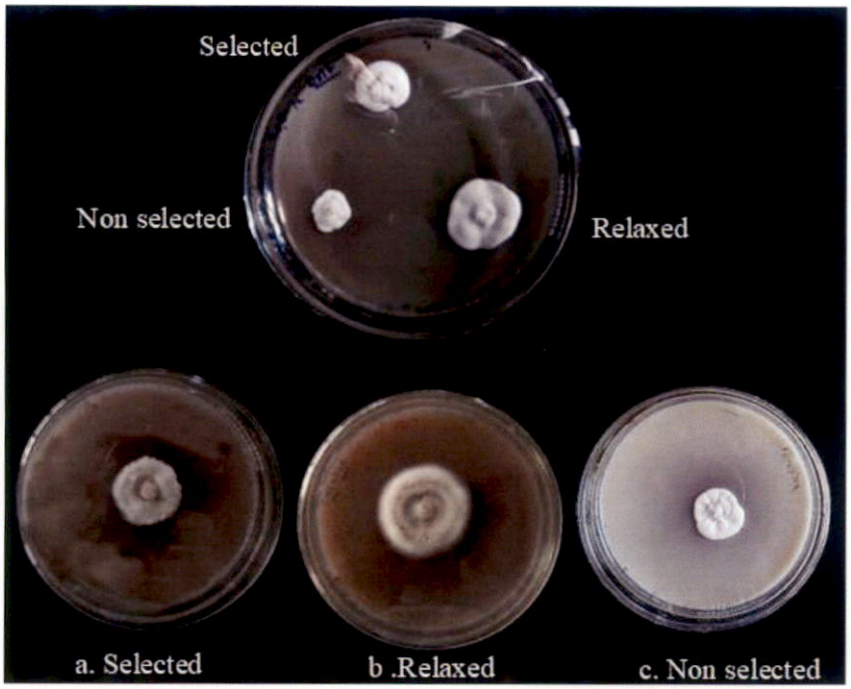


Plate 8. Colony growth of selected, relaxed and non-selected cultures of *Lecanicillium saksenae* on media poisoned with carbendazim 50% WP

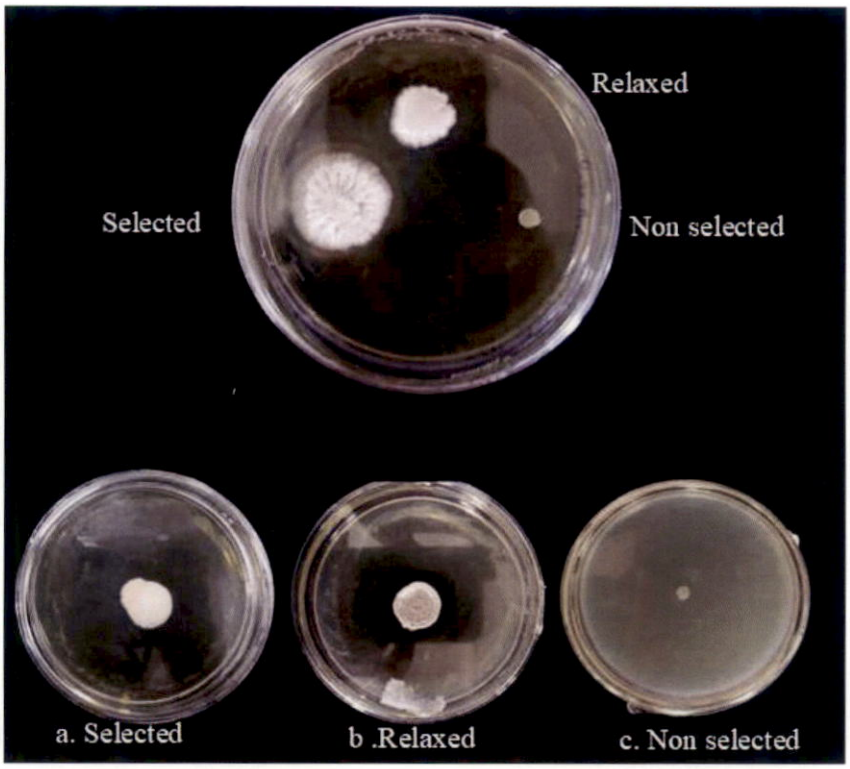


Plate 9. Colony growth of selected, relaxed and non-selected cultures of *Lecanicillium saksenae* on media poisoned with hexaconazole 5 % EC.

No germination was observed in non-selected culture. Colony growth, germination and sporulation was only observed in selected and relaxed culture, none was observed with non-selected culture at recommended dose.

Therefore, the carbendazim tolerant strain and hexaconazole tolerant strain of *L. saksenae* was further tested for molecular changes using REP-PCR.

4.4.3.1.2 L. lecanii

Comparative tolerance of selected, relaxed and non-selected culture of *L. lecanii* at recommended dose (0.2 %) and presented in Table 19.

4.4.3.1.2.1 Copper oxychloride 50% WP

No significant difference was observed in terms of radial growth between selected, relaxed and non-selected culture where growth of 3.09 cm, 2.91 and 3.03 cm was recorded respectively. Significant difference in sporulation was observed between selected, relaxed and non-selected culture where highest sporulation of 4.02×10^5 spores mL^{-1} recorded in non-selected culture. Exponential reduction in sporulation was observed in relaxed and selected culture where sporulation of 8.2×10^5 spores mL^{-1} and 6.5×10^4 spores mL^{-1} was observed respectively. No significant difference in germination was observed between selected relaxed and non-selected culture where germination per cent of 94.73 per cent, 94 per cent and 91 per cent was recorded respectively.

4.4.3.1.2.2 Azoxystrobin 23% SC

Highest radial growth (2.7 cm) was observed in non-selected culture followed by 2.25 cm growth in selected culture. Least growth (2.18 cm) was observed in relaxed culture. Significant difference in sporulation was observed between selected, relaxed and non-selected culture where highest sporulation of 4.73×10^5 spores mL^{-1} recorded in non-selected culture. Exponential reduction in sporulation was observed in relaxed and selected culture where sporulation of 6.8×10^4 spores mL^{-1} and 5.4×10^4 spores mL^{-1} was observed respectively.

Table 19. Comparative growth parameters of selected, relaxed and non-selected *Lecanicillium lecanii* in fungicides poisoned media at recommended dose

Culture	Copper oxy chloride 50% EC 0.2 %			Azoxystrobin 25% SC 0.1 %		
	Radial growth 21 DAI (cm)*	Sporulation (10^5 spores mL^{-1}) *	Germination (%)**	Radial growth 21 DAI (cm) *	Sporulation (10^5 spores mL^{-1}) **	Germination (%) *
Selected	3.09 (1.75)	0.65 $(0.79)^b$	94.73 (77.25)	2.25 $(1.5)^{ab}$	0.54 $(0.72)^b$	87.67 (69.89)
Relaxed	2.91 (1.71)	0.82 $(0.89)^b$	94.00 (76.7)	2.18 $(1.47)^b$	0.68 $(0.79)^b$	88.5 (71.35)
Non selected	3.03 (1.73)	4.02 $(1.94)^a$	91.00 (73.5)	2.70 $(1.64)^a$	4.73 $(1.97)^a$	87.33 (69.97)
CD (0.05)	NS	(0.429)	NS	(0.069)	(0.763)	NS
±S.Em	0.012	0.44	1.52	0.0923	0.749	0.860

NS- Non significant, DAI-Days After Inoculation, * Values in parentheses are square root transformed ** Values in parentheses are arc sine transformed.

No significant difference in germination was observed between relaxed selected and non-selected culture where germination per cent of 88.5 per cent, 87.67 per cent and 87.33 per cent was recorded respectively.

No improvement in growth parameters was observed in insecticide or fungicide induced strains of *L. lecanii*. Therefore, no pesticide tolerant strains could be developed and no molecular studies were carried out in this case.

4.4.3.2 Molecular Characters of Fungicide Tolerant L. saksenae

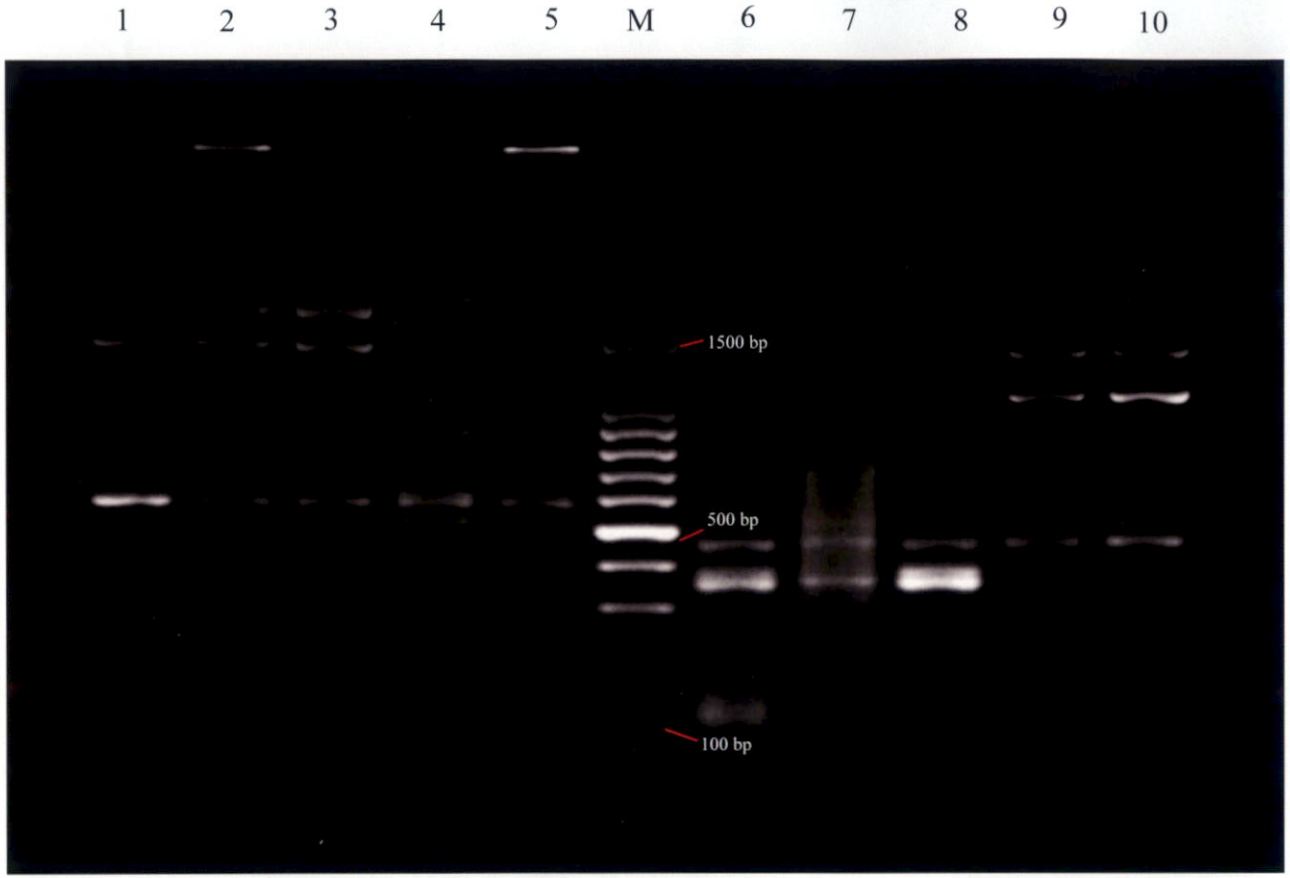
REP band profiles obtained by amplifying DNA from the tolerant as well as mother culture is presented below

PCR results of the non-selected *L. saksenae* [Ls(Ns)], selected carbendazim tolerant culture [LsC(S)], relaxed carbendazim tolerant culture [LsC(R)], selected hexaconazole tolerant culture [LSH(S)], relaxed hexaconazole tolerant culture [LsH(R)] is presented in Plate 10. The binary data analysed using NTSYS software package (version 2.1) and the similarity between each pair of DNA fingerprints as determined by Jaccard's coefficient is presented in Table 20.

Cluster analysis allowed the definition of two major groups (A and B) at similarity of 33 per cent (Fig. 1). Cluster A divided into two subclusters, where subcluster I had similarity of 62 per cent with subcluster II.

Based on Jaccard's coefficient, Ls(NS) was 41.667 per cent dissimilar to LsC(S) and LsC(R) and 37.5 per cent dissimilar to LsH(S) and LsH(R).

The similarity coefficient between improved cultures revealed that the cultures LsC(S) and LsC(R) were 79.16 per cent dissimilar to LsH(S) and LsH(R). LsC(S) was 100 per cent similar with LsC(R). LsH(S) and LsH(R) were also 100 per cent similar with each other, revealing that the genetic changes acquired due to tolerance induction were constant even after removal of selection pressure.



REP1R

Lane 1 - Non selected *L. saksenae*
Lane 2 - Selected carbendazim tolerant cult
Lane 3 - Relaxed carbendazim tolerant cult
Lane 4 - Selected hexaconazole tolerant cul
Lane 5 - Relaxed hexaconazole tolerant cul
Lane M - 100 bp Ladder

REP2I

Lane 6 - Non selected *L.saksenae*
Lane 7 - Selected carbendazim tolerant cult
Lane 8 - Relaxed carbendazim tolerant cult
Lane 9 - Selected hexaconazole tolerant cul
Lane 10 - Relaxed hexaconazole tolerant cul

Plate 10. Gel profiles of fungicide tolerant *L.saksenae* generated with REP primer

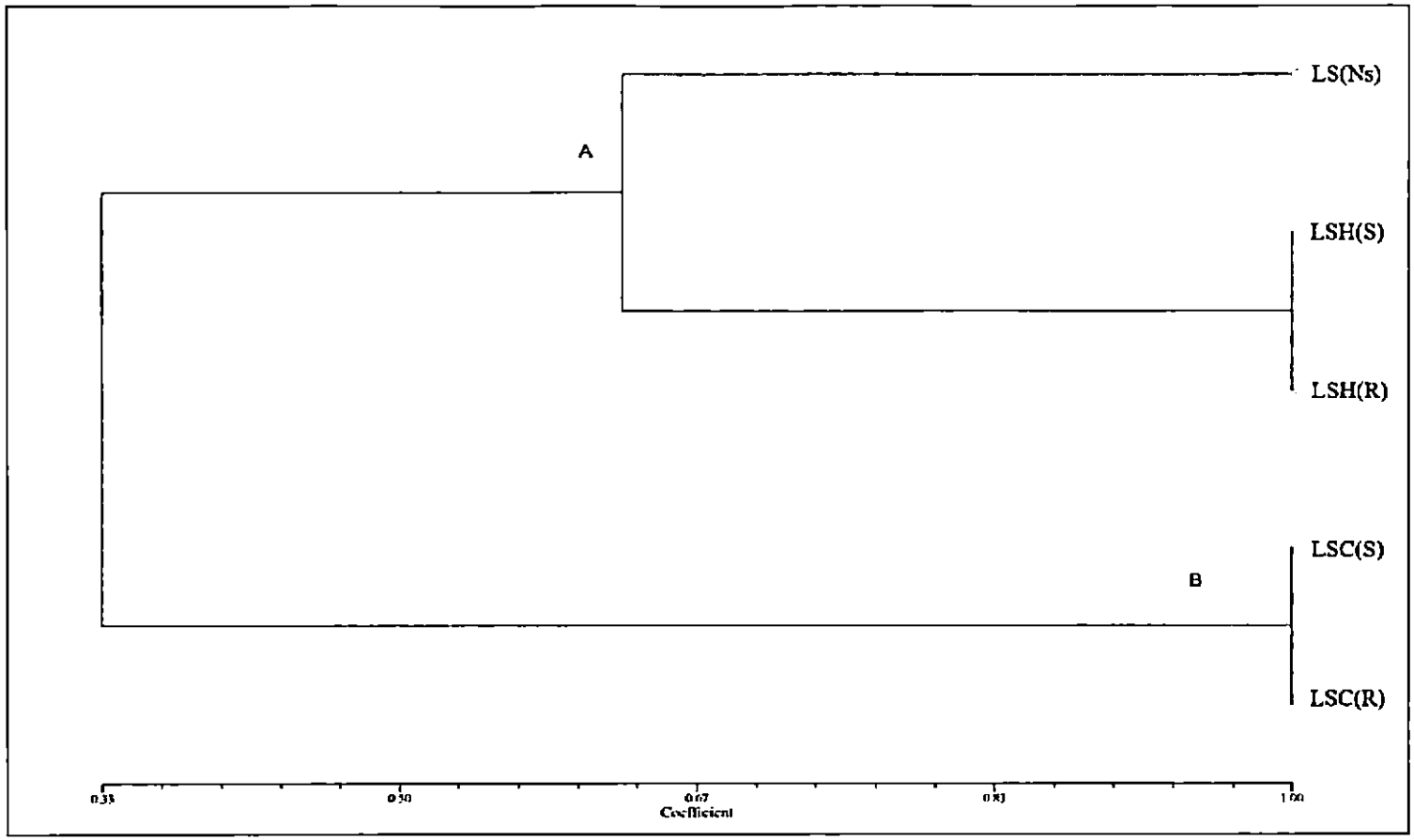


Fig. 1. Dendrogram from UPGMA cluster analysis of DNA banding profiles of *Lecanicillium saksenae* fungicide tolerant culture, generated with REP Primer. Similarity between the profiles was estimated with Jaccard's correlation coefficient. Ls(Ns)- Non-selected *L.saksenae,* LsC(S)- Selected carbendazim tolerant culture , LsC(R)- Relaxed carbendazim tolerant culture, LsH(S)- Selected hexaconazole tolerant culture, LsH(R)- Relaxed hexaconazole tolerant culture.

Table 20. Similarity matrix of non-selected and selected fungicide tolerant cultures of *Lecanicillium saksenae* based on Jaccard's coefficient

Type	Ls(NS)	LsC(S)	LsC(R)	LsH(S)	LsH(R)
Ls(NS)	1.000000				
LsC(S)	0.583333	1.000000			
LsC(R)	0.583333	1.000000	1.000000		
LsH(S)	0.625000	0.208333	0.20833	1.000000	
LsH(R)	0.625000	0.208333	0.208333	1.000000	1.000000

Ls(NS)- *L. saksenae* non-selected, LsC(S)- selected carbendazim tolerant culture, LsC(R)- relaxed carbendazim tolerant culture, LsH(S)- selected hexaconazole tolerant culture, LsH(R)- relaxed hexaconazole tolerant culture

4.5 ARTIFICIAL SELECTION FOR TEMPERATURE TOLERANCE

4.5.1 Highest Tolerant Temperature

Tolerance of the fungi to different temperature regimes of 30, 32, 34, 36, 38 and 40 °C, assessed in terms of colony growth rate, sporulation and germination is depicted below. Two factor analysis represented in Table 21 and 22 compares the growth rate and sporulation of each of the fungus under varying temperatures as well as the comparative tolerance between the species.

4.5.1.1 L. saksenae

4.5.1.1.1 Colony Growth Rate

The growth rate was found to be significantly reduced with increase in temperature (Plate 11) Highest growth rate of 4.51 mm day $^{-1}$ was recorded at 30 °C, followed by 3.85 mm day $^{-1}$ at 32°C. It was 1.72 mm day $^{-1}$ at 34 °C which was significantly higher to the rate at 36 °C (1.02 mm day^{-1}). Least growth of 0.68 mm day $^{-1}$ was observed at 38 °C and the fungus failed to grow at 40 °C. The corresponding growth rate was 6.03 mm day $^{-1}$ in control.

4.5.1.1.2 Sporulation

The spore count of *L. saksenae* was highest at 30°C (4.33 x 10^7 spores mL^{-1}) followed by the count in 32 °C eexposure (2.74 x 10^7 spores mL^{-1}). It was 1.32 x 10^7 spores mL^{-1} at 34 °C and 3.1 x 10^6 spores mL^{-1} at 36 °C, which varied significantly. No sporulation was observed at 38 and 40 °C. In the control the spore count was 4.67 x 10^7 spores mL^{-1}.

4.5.1.1.3 Germination

After 24 h of exposure, the rate of germination at 30 °C and 32 °C was on par (97.00 and 95.33 per cent respectively). At 34 °C there was 85.33 per cent germination which was significantly high compared to that at 36 °C and above. Germination rate was significantly less at 36 °C and 38 °C (53.47 and 22.67 per cent).

Table 21. Effect of temperature on growth and sporulation of *Lecanicillium saksenae* and *Lecanicillium lecanii*

Temperature (°C) (8h @test temperature + 16 h @26 °C)	Growth rate (mm day^{-1}) ± S.E			Spore count (10^7 spores mL^{-1}) 21 DAI		
	L. saksenae	*L. lecanii*	Mean	*L. saksenae*	*L. lecanii*	Mean
30	4.51 ± 0.091^{bA}	2.58 ± 0.12^{bB}	3.548	4.33 $(2.31)^{bA}$	1.06 $(1.43)^{bB}$	0.731
32	3.85 ± 0.059^{cA}	1.31 ± 0.065^{cB}	2.58	2.74 $(1.93)^{cA}$	0.74 $(1.32)^{bB}$	0.525
34	1.72 ± 0.031^{dA}	0.92 ± 0.027^{dB}	1.319	1.32 $(1.52)^{dA}$	0.03 $(1.02)^{cB}$	0.251
36	1.02 ± 0.037^{eA}	0 ± 0^{eB}	0.51	0.31 $(1.14)^{eA}$	0 $(0.71)^{dB}$	0.112
38	0.68 ± 0.046^{fA}	0 ± 0^{eB}	0.34	0 $(0.71)^{fA}$	0 $(0.71)^{dA}$	0.094
40	0 ± 0^{gA}	0 ± 0^{eB}	0	0 $(0.71)^{fA}$	0 $(0.71)^{dA}$	0
Control (26 °C)	6.03 ± 0.098^{aA}	3.89 ± 0.063^{aB}	4.963	4.67 $(2.35)^{aB}$	5.01 $(2.45)^{Aa}$	1.018
Mean	2.545	1.244		1.91 (1.61)	0.98 (1.32)	
For comparing mean of	S. Em ±	CD (0.05)		S. Em ±	CD (0.05)	
Temperature (A)	0.042	(0.121)		0.056	(0.163)	
L. lecanii and L. saksenae (B)	0.022	(0.065)		0.03	(0.087)	
A x B	0.059	(0.171)		0.079	(0.23)	

Values sharing same alphabets in superscript are statistically on par based on ANOVA. Upper case alphabets depict statistical significance between fungi and lowercase alphabets depicts the same across the exposure duration. Values in parenthesis are square root transformed.

Table 22. Effect of temperature on germination of *Lecanicillium saksenae* and *Lecanicillium lecanii*

Temperature (8h test +16h @ 26 °C)	Germination after 24 h (%)			Germination after 48 h (%)		
	L. saksenae	*L. lecanii*	Mean	*L. saksenae*	*L. lecanii*	Mean
30	97.00 (81.85)^{bA}	66.33 (54.54)^{bB}	81.66	100 (90)^{aA}	77.33 (61.58)^{bB}	88.66
32	95.33 (77.72)^{bA}	59.00 (50.19)^{bB}	77.16	100 (90)^{aA}	66.67 (54.74)^{cB}	83.33
34	85.33 (67.55)^{cA}	20.00 (26.49)^{cB}	52.66	91.67 (73.26)^{bA}	28.00 (31.93)^{dB}	59.83
36	53.47 (46.99)^{dA}	12.00 (20.17)^{dB}	32.73	85.00 (67.26)^{bA}	13.33 (21.31)^{eB}	49.16
38	22.67 (28.42)^{eA}	2.00 (4.73)^{eB}	12.33	33.33 (35.26)^{cA}	2.33 (5.11)^{fB}	17.83
40	18.00 (24.99)^{fA}	0 (0.71)^{fB}	9	24.33 (29.53)^{dA}	0 (0.71)^{gB}	12.16
Control (26 °C)	100 (90)^{aA}	98 (83.44)^{aB}	99	100 (90)^{aA}	100 (90)^{aA}	100
Mean	67.40	36.762		76.33	41.09	

For comparing mean of	S. Em ±	CD (0.05)	S. Em ±	CD (0.05)
UV exposure time (A)	1.539	4.480	1.119	3.259
L. lecanii and L. saksenae (B)	0.822	2.395	0.598	1.742
A x B	2.176	6.336	1.583	4.608

Figures in the parentheses are angular transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA. Upper case alphabets depict statistical significance between fungi and lowercase alphabets depicts the same across the exposure duration.

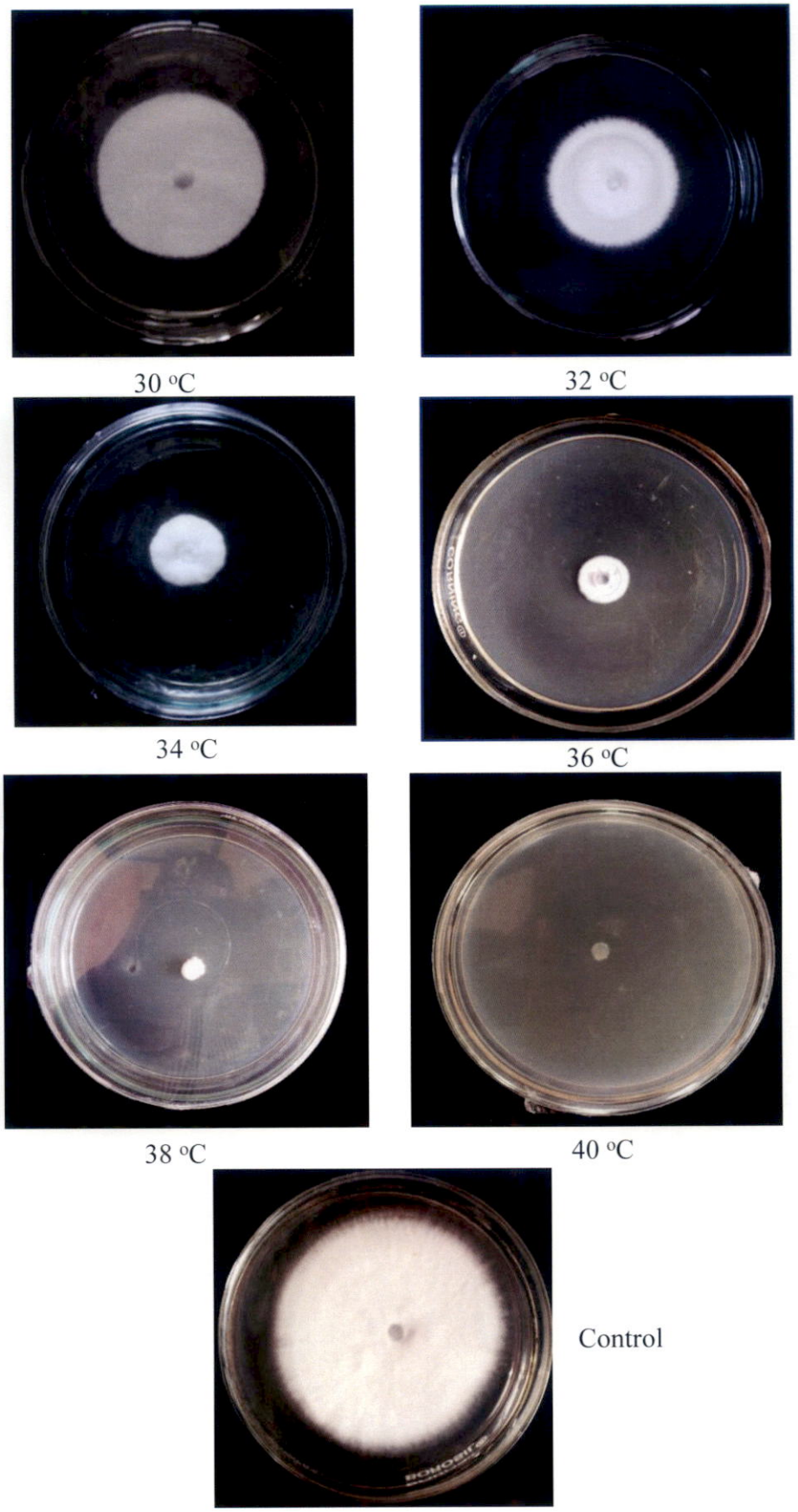


Plate 11. Colony growth of *Lecanicillium saksenae* at different temperature regimes (8h)

Least germination (18 per cent) was observed at 40 °C. In control there was 100 per cent germination. By 48 h all the conidia exposed to 30 and 32 °C germinated. At 34 °C, there was 91.67 per cent germination which was significantly high when compared to 36 °C exposure (85 per cent). The germination rate was significantly low at 38 and 40 °C (33.33 per cent and 24.33 per cent respectively). In control, 100 per cent germination was observed.

4.5.1.2 L. lecanii

4.5.1.2.1 Colony Growth

Growth rate of *L. lecanii* was significantly affected by all the test temperatures (Plate 12). Growth rate of 2.58 mm day $^{-1}$ was recorded at 30 °C followed by 1.31 mm day $^{-1}$ at 32 °C and 0.92 mm day^{-1} at 34 °C. No growth was recorded at 36° C and above. Growth rate of 3.89 mm day $^{-1}$ was recorded in control.

4.5.1.2.1 Sporulation

Sporulation of *L. lecanii* was significantly affected by all the test temperature. Sporulation of 1.06 x 10^7 spores mL^{-1} was recorded at 30 °C followed by 7.4 x 10^6 spores mL^{-1} at 32 °C. Exponential decrease in sporulation (3 x 10^5 spores mL^{-1}) was observed at 34 °C. No sporulation was observed at 36 °C and above. In the control, the count was 5.01 x 10^7 spores mL^{-1}.

4.5.1.2.3 Germination

After 24 h exposure, germination was found to be significantly affected in all the test temperatures. At 30 and 32 °C, it was 66.33 per cent and 59.00 per cent which were on par. At 34 °C the rate was only 20.00 per cent which was significantly lower. The germination rate further reduced significantly to 12 per cent at 36 °C and 2 per cent at 38 °C. Spore viability was decreased to zero at 40 °C. In control 98 per cent germination was recorded.

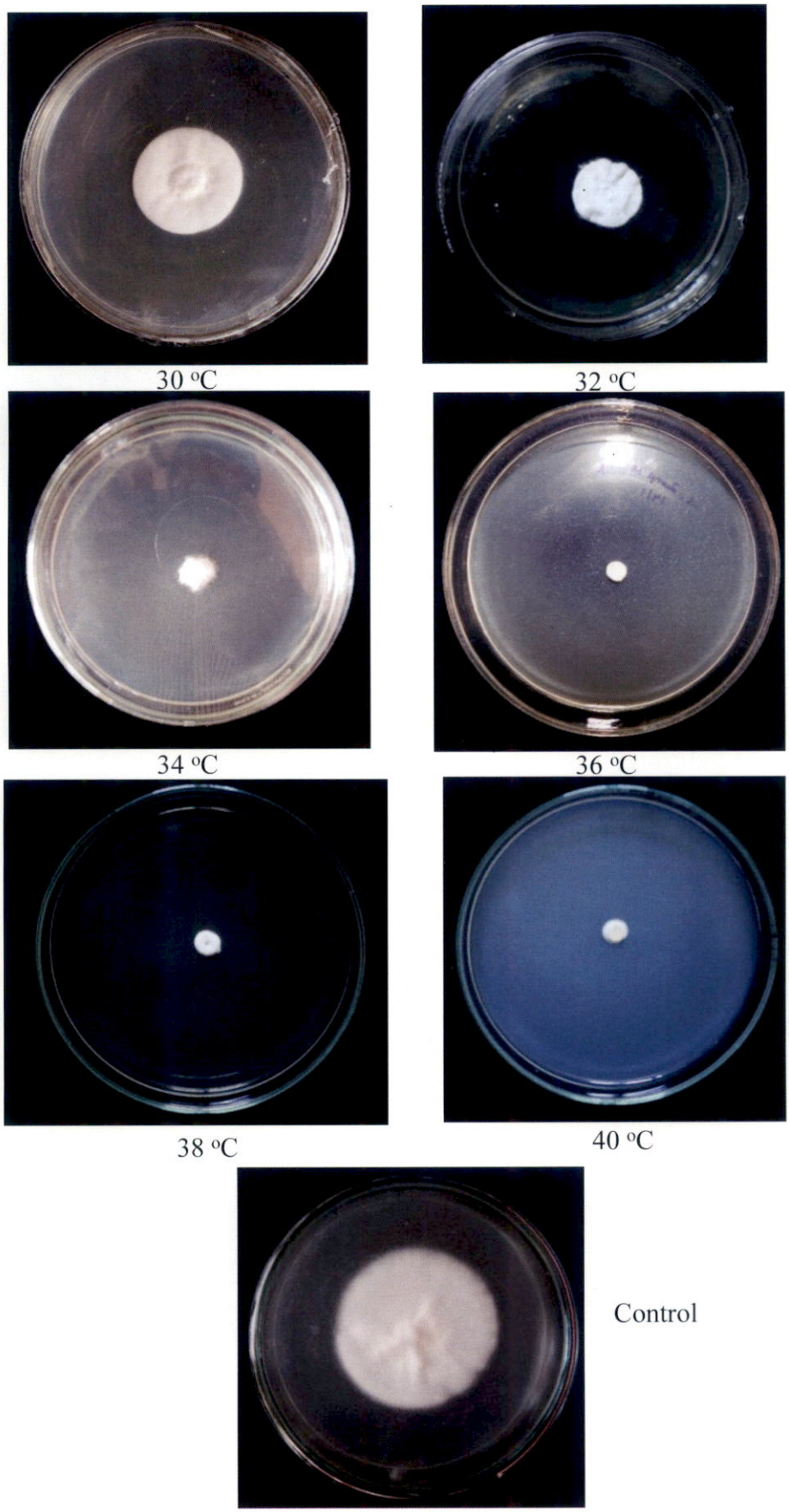


Plate 12. Colony growth of *Lecanicillium lecanii* at different temperature regimes (8h)

After 48h, the highest rate observed was 77.33 per cent at 30 °C, which was followed by 66.67 per cent at 32 °C. There was only 28.00 per cent and 13.33 per cent germination at 34 °C and 36 °C, respectively which were statistically different from each other. The rate was negligible (2.33 per cent) while it was zero at 40 °C.

4.5.1.3 Comparative Thermotolerance of L. saksenae and L. lecanii

4.5.1.3.1 Colony Growth

Comparative analysis of thermotolerance data revealed that growth rate of *L. saksenae* was significantly more compared to *L. lecanii* at varying temperatures (Table 21). Growth of 4.51, 3.85 and 1.72 mm day $^{-1}$ was recorded at 30, 32 and 34 °C respectively in *L. saksenae*, while it was significantly low in *L. lecanii* (2.58, 1.31 and 0.92 mm day^{-1} respectively). Colony growth completely terminated at and above 36 °C in *L. lecanii* whereas the growth rate was sustained to 1.02 mm day $^{-1}$and 0.68 mm day $^{-1}$ in *L. saksenae.*

4.5.1.3.2 Sporulation

In all the test temperatures, sporulation was significantly more in *L. saksenae* compared to *L. lecanii.* Sporulation of 4.33 x 10^7 spores mL^{-1}, 2.74 x 10^7 spores mL^{-1} and 1.32 x 10^7 spores mL^{-1} was recorded at 30, 32 and 34 °C respectively which was significantly more than growth in *L. lecanii* where the count was 1.06 x 10^7 spores mL^{-1}, 7.4 x 10^6 spores mL^{-1} and 3.0 x 10^5 spores mL^{-1}, respectively. Sporulation completely terminated at 36 °C in *L. lecanii* whereas sporulation of 3.1 x 10^6 spores mL^{-1} was observed at *L. saksenae.* Growth was completely terminated at 38 and 40 °C in both the fungi. In the control, sporulation of 5.01 x 10^7 spores mL^{-1} was recorded in *L. lecanii* which significantly more than sporulation (4.67 x 10^7 spores mL^{-1}) in *L. saksenae.*

4.5.1.3.3 Conidial Germination

After 24h, all the test temperatures showed significantly higher germination rate in *L. saksenae* compared to *L. lecanii* (Table 22). At 30, 32 and

34 °C conidial germination rate was 97.00, 95.33 and 85.33 per cent respectively. in *L. saksenae* which was significantly higher compared to *L. lecanii* with the rates 66.33, 59.00 and 20 per cent respectively. At 36 and 38 °C, germination rate was 53.47 and 22.67 per cent *L. saksenae* and 12.00 and 2.00 per cent in *L. lecanii*. At 40 °C, 18.00 per cent germination was recorded in *L. saksenae* and no germination was observed in *L. lecanii*.

After 48h also, *L. saksenae* showed significantly higher germination compared to *L. lecanii*. At 30 and 32 °C, 100 per cent germination was recorded in *L. saksenae* which was significantly higher to *L. lecanii* where the rate was 77.33 per cent and 66.67 per cent. There was 91.67, 85.00 and 33.33 per cent germination in *L. saksenae* at 34, 36 and 38 °C respectively while the corresponding rate was 28.00, 13.33 and 2.33 per cent respectively in *L. lecanii*. At 40 °C, 24.33 per cent germination was recorded in *L. saksenae* while none of the conidia germinated in *L. lecanii*.

The above results revealed that the Highest Tolerant Temperature (HTT) for *L. saksenae* is 36 °C and for *L. lecanii* it is 34 °C where germination, sporulation and growth were observed.

4.5.2 Induced Temperature Tolerance

The growth parameters of *L. saksenae* and *L. lecanii* induced to HTT by subculturing in 10 successive generations is detailed below.

4.5.2.1 L saksenae

Mycelial growth, sporulation and germination observed for seven generations is presented in Table 23. The growth and sporulation terminated after seventh generation.

4.5.2.1.1 Colony Growth

In the first three generations there was no significant difference among the mean colony diameter. It was 1.58 cm. 1.6 cm. and 1.52 cm in the first, second

Table 23. Growth parameters of *Lecanicillium saksenae* at highest tolerant temperature (36 °C)

Generation	Colony growth (cm)* 21 DAI	Spore count (10^6 spores mL^{-1})* 21 DAI	Germination (%)**	
			24 h	48 h
I	1.58 (1.44)[a]	3.17 (1.89)[a]	43.00 (40.83)	74.20 (60.13)
II	1.60 (1.45)[a]	3.03 (1.86)[a]	42.40 (40.6)	76.20 (61.33)
III	1.52 (1.42)[a]	0.80 (1.14)[b]	36.40 (37.07)	71.20 (57.73)
IV	1.22 (1.31)[b]	0.71 (1.1)[b]	42.80 (40.83)	75.40 (60.48)
V	1.10 (1.26)[b]	0.45 (0.96)[c]	37.60 (37.79)	73.80 (59.43)
VI	0.92 (1.19)[b]	0.14 (0.8)[d]	40.80 (39.63)	78.80 (62.75)
VII	0.00 (0.71)[C]	0.00 (0.71)[d]	-	-
CD (0.05)	(0.109)	(0.245)	NS	NS
S.Em±	0.213	0.506	5.962	10.801

DAI- Days after inoculation; * Values in parentheses are square root transformed** Values in parentheses are arc sine transformed.

and third generation respectively. In the fourth and fifth generations there was significant decrease in growth. The mean colony diameter was 1.22 cm and 1.10 cm respectively. In the sixth generation, growth was negligible (0.92 cm) and no growth was observed in the seventh generation.

4.5.2.1.2 Sporulation

There was no significant variation among the first and second generations. The spore load was 3.17 x 10^6 spores mL^{-1} and 3.03x 10^6 spores mL^{-1}. Exponential reduction in spore count was noted from the third generation onwards. It was 8 x 10^5 spores mL^{-1} in the third and 7.10 x 10^5 spores mL^{-1}, in the fourth which were statistically similar. Sporulation observed in the fifth and sixth generations was 4.5 x 10^5 spores mL^{-1} and 1.40 x 10^5 spores mL^{-1} which were statistically different from each other. No sporulation was observed in seventh generation.

4.5.2.1.3 Germination

After 24 h there was no significant variation in the germination rate, across the first six generations. Conidial germination varied between 43 per cent in the first to the lowest of 36.4 per cent in the third. No significant difference was observed even after 48h, across the six generations. Conidial germination varied between the highest rate of 78.8 per cent in the first to the lowest of 71.2 per cent in third generation.

Therefore, it is inferred that *L. saksenae* can tolerate the HTT (36 °C) up to sixth generation.

4.5.2.2 L. lecanii

Mycelial growth, sporulation and germination observed for five generations is presented in Table 24.

4.5.2.2.1 Colony Growth

In the first generation, the colony diameter was 1.18 cm which was statistically on par with that noted in the second generation (1.16 cm).

Table 24. Growth parameter of *Lecanicillium lecanii* at highest tolerant temperature (34 °C)

Generation	Colony growth (cm)* 21 DAI	Spore count (10^6 spores mL^{-1}) * 21DAI	Germination (%)**	
			24 h	48 h
I	1.18 (1.3)[a]	2.42 (1.71)[a]	21.00 (27.19)	24.60 (29.64)
II	1.16 (1.29)[a]	0.49 (0.99)[b]	22.40 (28.19)	28.40 (32.18)
III	1.02 (1.23)[a]	0.18 (0.81)[b]	19.60 (26.2)	24.8 (29.84)
IV	1.10 (1.26)[a]	0.01 (0.71)[b]	21.80 (27.83)	23.60 (29.00)
V	0 (0.71)[a]	0 (0.71)[b]	-	-
CD (0.05)	(0.140)	(0.367)	NS	NS
S.Em ±	0.225	0.459	4.266	5.135

DAI-Days after inoculation * Values in parentheses are square root transformed
** Values in parentheses are arc sine transformed.

No significant difference in growth was observed during the third and fourth generations (1.02 cm and 1.1 cm). No growth was observed in the fifth generation

4.5.2.2.2 Sporulation

Highest sporulation of 2.42 x 10^6 spores mL^{-1} was observed in the first generation. Thereafter, there was an exponential decrease from the second generation onwards. It was 4.90 x 10^5 spores mL^{-1} in the second and 1.80 x 10^5 spores mL^{-1}, in the third which were statistically similar to each other. Sporulation observed in the fourth generation was 1 x 10^4 spores mL^{-1} while there was no sporulation in the fifth generation,

4.5.2.2.3 Germination

No significant variation was observed in the germination rate after 24h, across five generations. Conidial germination was only 21.00 and 22.40 per cent in the first and second generations, while it was 19.60 per cent and 21.80 per cent in the third and fourth generations respectively.

After 48h, no significant variation was noted across the five generations. Germination rate was 24.6 and 28.4 per cent in the first and second generations, while it was 24.9 per cent and 23.6 per cent in the third and fourth generations. The growth and sporulation terminated at fifth generation.

Therefore, it is inferred that *L. lecanii* can tolerate HTT (34 °C) up to the fourth generation.

There was no phenotypic variation after successive subculturing of *L. saksenae* in seven generations, and *L. lecanii* in five generations. Therefore, no temperature tolerant strain could be developed in both the fungi.

4.6 TOLERANCE OF *L. saksenae* and *L. lecanii* To UV RADIATION

Tolerance of *L. saksenae* and *L. lecanii* to UVA and UVB assessed in terms of colony growth, sporulation and germination after exposure is detailed below.

4.6.1 Tolerance of *L. saksenae* and *L. lecanii* to UVA

4.6.1.1 Effect of UVA on Colony Growth

Colony growth of *L. saksenae* and *L. lecanii* was significantly affected by UVA (Table 25).

4.6.1.1.1 L. saksenae

Growth of *L. saksenae* after exposing to UVA for 10, 20 and 30 min did not vary significantly among each other (Plate 13). The mean colony diameter was 2.28 mm, 2.25 mm and 2.21 mm respectively. After 1h exposure, the growth was 1.95 mm which was significantly higher to the growth noted after 2h and 3h (1.53 mm and 1.22 mm). Least growth (0.65 mm) was exhibited after 4 h exposure. The growth observed in unexposed control was 2.64 mm.

4.6.1.1.2 L. lecanii

The growth recorded in 10, 20 and 30 min exposure did not vary significantly, with the mean colony diameter of 1.71 mm, 1.67 mm and 1.68 mm, respectively (Plate 14). Growth reduction was noted in 1h exposure (1.57 mm) which was significantly higher to that noted in 2h (1.13 mm) and 3h (0.83 mm) exposure. Least growth (0.42 mm) was recorded in 4h exposure. The corresponding growth in unexposed control was 2.23 mm.

4.6.1.2 Effect of UVA on Sporulation

Spore count of *L. saksenae* and *L. lecanii* exposed to UVA, taken on the 21st day is represented in Table 25.

Table 25. Effect of UVA exposure on growth and sporulation of *Lecanicillium saksenae* and *Lecanicillium lecanii*

Exposure time	Colony growth (mm) ± SE at 48 h			Spore count (10^7 spores mL^{-1}) 21 DAI		
	L. saksenae	*L. lecanii*	Mean	*L.saksenae*	*L. lecanii*	Mean
10 min	2.28 ± 0.122^{bA}	1.71 ± 0.015^{bB}	2.00	$5.31\ (2.5)^{aA}$	$2.79\ (1.95)^{bB}$	4.05
20 min	2.25 ± 0.148^{bA}	1.67 ± 0.013^{bB}	1.96	$5.09\ (2.46)^{aA}$	$2.68\ (1.92)^{bB}$	3.88
30 min	2.21 ± 0.124^{bA}	1.68 ± 0.016^{bB}	1.94	$5.12\ (2.47)^{aA}$	$2.66\ (1.91)^{bB}$	3.89
1h	1.95 ± 0.037^{cA}	1.57 ± 0.014^{cB}	1.76	$3.68\ (2.16)^{bA}$	$1.42\ (1.51)^{cB}$	2.55
2h	1.53 ± 0.021^{dA}	1.13 ± 0.012^{dB}	1.33	$3.83\ (2.18)^{bA}$	$1.27\ (1.48)^{cB}$	2.55
3h	1.22 ± 0.007^{eA}	0.83 ± 0.028^{eB}	1.02	$1.98\ (1.72)^{cA}$	$1.31\ (1.49)^{cB}$	1.64
4h	0.65 ± 0.018^{fA}	0.42 ± 0.02^{fB}	0.53	$1.72\ (1.64)^{cA}$	$1.34\ (1.52)^{cB}$	1.53
Unexposed (control)	2.64 ± 0.018^{aA}	2.23 ± 0.019^{aB}	2.43	$5.53\ (2.55)^{aA}$	$5.11\ (2.46)^{aA}$	5.32
Mean	1.84	1.4		4.03	2.32	
For comparing mean of	S. Em ±	CD (0.05)		S. Em ±	CD (0.05)	
UV exposure time (A)	0.045	(0.12)		0.107	(0.311)	
L. lecanii and L. saksenae (B)	0.022	(0.06)		0.054	(0.156)	
A x B	0.060	(0.17)		0.152	NS	

Values sharing same alphabets in superscript are statistically on par based on ANOVA. Upper case alphabets depict statistical significance between fungi and lowercase alphabets depicts the same across the exposure duration.

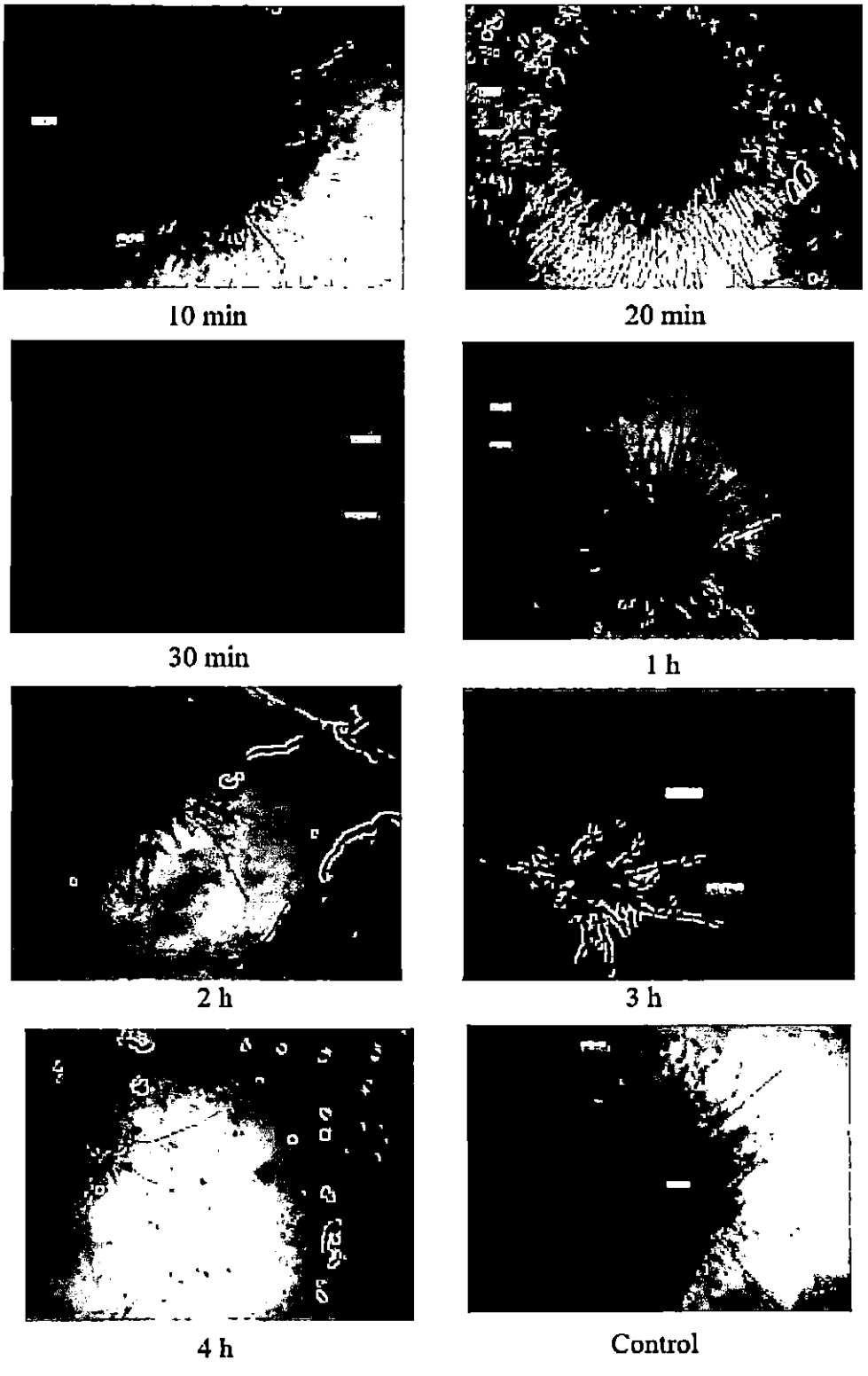


Plate 13. Colony growth of *Lecanicillium saksenae* exposed to UVA (48h, 40X)

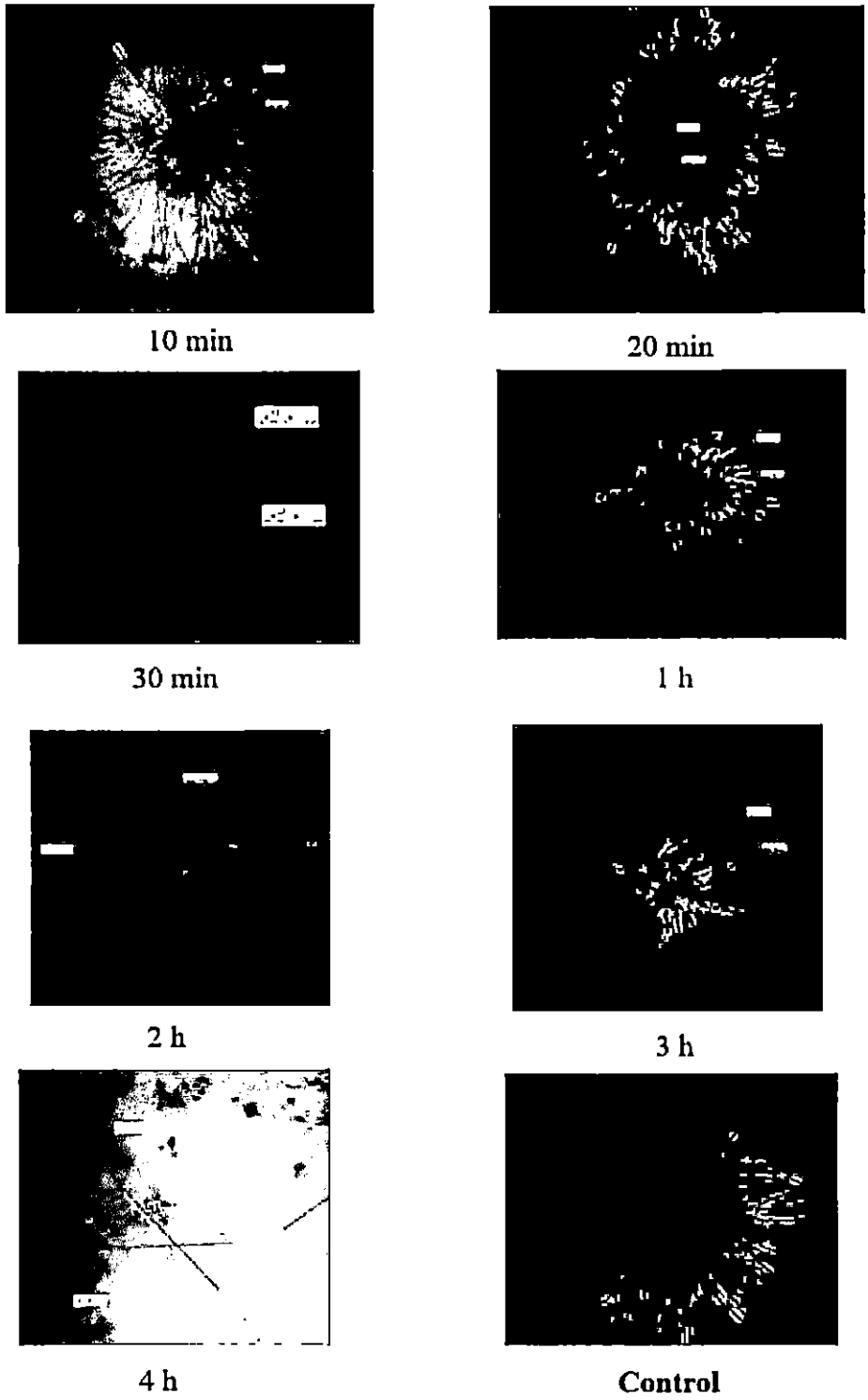


Plate 14. Colony growth of *Lecanicillium lecanii* exposed to UVA (48h, 40X)

4.6.1.2.1 L. saksenae

The spore count noted in 10, 20 and 30 min exposure, did not vary significantly from the control. The count ranged from 5.09 x 10^7 spores mL^{-1} to 5.53 x 10^7 spores mL^{-1}. Spore count at 1h and 2h exposure was 3.68 x 10^7 spores mL^{-1} and 3.83 x 10^7 spores mL^{-1} which were on par with each other. Spore count of 1.98 x 10^7 spores mL^{-1} and 1.72 x 10^7 spores mL^{-1} observed at 3h and 4h of exposure were lower and were on par.

4.6.1.2.2 L. lecanii

Sporulation of *L. lecanii* was significantly affected after UV exposure. Spore count of 2.79 x 10^7 spores mL^{-1} was recorded at 10 min exposure which was significantly lower than control (5.11 x 10^7 spores mL^{-1}). No significant decrease in sporulation was observed among 20 and 30 min exposure (2.68 x 10^7 spores mL^{-1} and 2.66 x 10^7 spores mL^{-1}). At 1, 2, 3 and 4 h of exposure, the count was 1.42 x 10^7 spores mL^{-1}, 1.27 x 10^7 spores mL^{-1}, 1.31 x 10^7 spores mL^{-1} and 1.34 x 10^7 spores mL^{-1} respectively which were on par with each other.

4.6.1.3 Effect of UVA on Germination

Conidial germination of the fungi recorded after 24 and 48 h exposure, expressed as percentage is depicted in Table 26.

4.6.1.3.1 L. saksenae

After 24h, the rate of germination was 34.00 and 30.33 per cent in 10 and 20 min exposure, which was significantly more compared to others. In 30 min exposure the rate of germination was 19.00 per cent which was superior to 1, 2, 3 and 4 h exposure where the rate of germination ranged between 9.33 and 12.00 per cent. In unexposed control there was 97.67 per cent germination.

After 48 h, there was 44 per cent and 39.67 per cent germination in 10 and 20 min exposure.

Table 26. Effect of UVA exposure on germination of *Lecanicillium saksenae* and *Lecanicillium lecanii*

Exposure time	Germination (%) @ 24 h			Germination (%) @ 48 h		
	L. saksenae	*L. lecanii*	Mean	*L. saksenae*	*L. lecanii*	Mean
10 min	34.00 (35.6)^{bB}	47.33 (43.47)^{bA}	40.67	44.00 (41.53)^{bB}	73.33 (58.96)^{bA}	58.67
20 min	30.33 (33.41)^{bB}	36.33 (37.06)^{cA}	33.33	39.67 (39.03)^{bB}	46.67 (43.09)^{cA}	43.17
30 min	19.00 (25.80)^{cB}	25.00 (29.97)^{dA}	22.00	25.67 (30.39)^{cB}	31.33 (34.02)^{dA}	28.50
1h	12.00 (20.26)^{dA}	12.33 (20.40)^{eA}	12.17	18.67 (25.6)^{dA}	18.00 (24.94)^{eA}	18.33
2h	11.33 (19.62)^{dA}	12.00 (20.17)^{eA}	11.67	16.00 (23.57)^{dA}	15.67 (23.21)^{eA}	15.83
3h	10.33 (18.69)^{dA}	7.67 (15.95)^{fB}	9.00	17.33 (24.5)^{dA}	14.67 (22.45)^{eB}	16.00
4h	9.33 (17.75)^{dA}	7.33 (15.66)^{fB}	8.33	15.33 (22.97)^{dA}	13.33 (21.23)^{eB}	14.33
Unexposed (control)	97.67 (82.98)^{aB}	100.00 (90.00)^{aA}	98.83	100.00 (90.00)^{aA}	100.00(90.00)^{aA}	100.00 (90.00)
Mean	28.00	31.00		34.58	39.13	

For comparing mean of	S. Em ±	CD (0.05)	S. Em ±	CD (0.05)
UV exposure time (A)	1.10	(3.17)	1.04	(3.01)
L. lecanii and L. saksenae (B)	0.55	(1.58)	0.52	(1.50)
A x B	1.55	(4.48)	1.48	(4.26)

Figures in the parentheses are arc sin root transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA. Upper case alphabets depict statistical significance between fungi and lowercase alphabets depicts the same across the exposure duration.

The rate was 25.67 per cent at 30 min exposure duration, which was significantly higher to 1, 2, 3 and 4 h exposures, where the rate of germination was 18.67, 16.00, 17.33 and 15.33 per cent which were on par with each other. In the control, there was 100 per cent germination.

4.6.1.3.2 L. lecanii

After 24 h, in 10 min exposure, the rate of germination was 47.33 per cent was recorded followed by 36.33 per cent in 20 min exposure and 25 per cent in 30 min exposure which were significantly different from each other. In 1h and 2h exposure, the rate of germination was 12.33 per cent and 12.00 per cent which were on par with each other. No significant difference in germination was observed in 3h and 4h exposure (7.67 and 7.33 per cent respectively). In the unexposed control, there was 100 per cent conidial germination.

After 48 h, there was 73.33 per cent germination in 10 min exposure time, followed by 46.67 per cent in 20 min and 31.33 per cent in 30 min exposure, which was significantly different from each other. At 1, 2, 3 and 4 h exposures the rate of germination was 18.00, 15.67, 14.67 and 13.33 per cent respectively, which were on par with each other.

4.6.1.4 Comparative Tolerance of L. saksenae and L. lecanii to UVA

L. saksenae exhibited higher colony growth than *L. lecanii*, in all the exposures. Colony growth ranged between 2.28 to 2.21 mm at 10, 20 and 30min exposure duration in *L. saksenae* which was significantly higher than that observed in *L. lecanii* where colony growth ranged between 1.71 to 1.68 mm. At 1, 2, 3 and 4 h exposure, colony growth ranged between 0.65mm to 1.95mm in *L. saksenae* whereas it was significantly lower in *L. lecanii* (0.42 to 1.57mm).

Sporulation was significantly more in *L. saksenae* compared to *L. lecanii.* The spore count ranged between 5.31x 10^7 spores mL^{-1} to 5.12 x 10^7 spores mL^{-1} at 10 min, 20 min and 30 min exposure duration which was significantly higher

than that observed in *L. lecanii* where sporulation ranged between 2.79 x 10^7 spores mL^{-1} to 2.66 x 10^7 spores mL^{-1}. At 1h, 2h, 3h and 4h exposure, *L. saksenae* exhibited higher sporulation (3.68 x 10^7 spores mL^{-1} to 1.72 x 10^7 spores mL^{-1}) than *L. lecanii* (1.42 x 10^7 spores mL^{-1} to 1.34 x 10^7 spores mL^{-1}). In unexposed control no significant difference was observed between *L. saksenae* and *L. lecanii* where sporulation of 5.53 x 10^7 spores mL^{-1} and 5.11 x 10^7 spores mL^{-1} was observed.

At 10, 20 and 30 min of exposure *L. lecanii* exhibited significantly higher germination after 48 h (73.33, 46.67 and 31.33 per cent respectively) compared to *L. saksenae* (44.00 39.67 and 25.67 per cent). As the exposure duration increased to 1h and 2h the germination was observed to be on par in both the fungi. Further increase in exposure to 3h and 4h, *L. saksenae* exhibited significantly more germination (17.33 and 15.33 per cent respectively) compared to *L. lecanii* (14.67 and 13.33 per cent respectively).

4.6.2 Tolerance of *L. saksenae* and *L. lecanii* to UVB Radiation

4.6.2.1 Effect of UVB on Colony Growth

Growth of fungus recorded at 48 h after exposure is illustrated in Table 27. In both the fungi growth was found to be significantly affected by UVB exposure.

4.6.2.1.1 L. saksenae

In 10 min exposure, the colony diameter was 2.224 mm which was significantly higher to all other treatments (Plate 15). In 20 and 30 min exposures the growth noted was 2.139 mm and 2.058 mm which were on par with each other. Growth was significantly less (1.34 mm) in 1h and 2h exposures (0.938 mm) which were significantly different from each other. At 3h exposure growth of 0.583 mm was observed. Least growth (0.363 mm) was observed in 4 h exposure. The growth observed in unexposed control was 2.554 mm.

Table 27. Effect of UVB exposure on growth and sporulation of *Lecanicillium saksenae* and *Lecanicillium lecanii*

Exposure time	Colony growth (mm) ± SE @ 48h			Spore count (10^7 spores mL^{-1}) 21DAI		
	L. saksenae	*L. lecanii*	Mean	*L. saksenae*	*L. lecanii*	Mean
10 min	2.224 ± 0.058^{bA}	1.667 ± 0.079^{bB}	1.95	$5.46\ (2.54)^{aA}$	$2.21\ (1.79)^{bB}$	3.72
20 min	2.139 ± 0.031^{bA}	1.630 ± 0.055^{bB}	1.88	$5.23\ (2.49)^{aA}$	$2.13\ (1.77)^{bB}$	3.64
30 min	2.058 ± 0.042^{bA}	1.623 ± 0.058^{bB}	1.84	$5.15\ (2.47)^{aA}$	$2.05\ (1.75)^{bB}$	3.76
1h	1.340 ± 0.070^{cA}	1.070 ± 0.089^{cB}	1.21	$2.38\ (1.83)^{bA}$	$1.13\ (1.45)^{cB}$	1.75
2h	0.938 ± 0.034^{dA}	0.828 ± 0.053^{dB}	0.91	$2.46\ (1.86)^{bA}$	$1.19\ (1.48)^{cB}$	1.83
3h	0.583 ± 0.042^{eA}	0.405 ± 0.032^{eB}	0.56	$1.80\ (1.66)^{bA}$	$1.10\ (1.44)^{cB}$	1.45
4h	0.363 ± 0.025^{fA}	0.253 ± 0.048^{fB}	0.33	$1.67\ (1.63)^{bA}$	$1.06\ (1.44)^{cB}$	1.37
Unexposed (control)	2.554 ± 0.067^{aA}	2.418 ± 0.026^{aB}	2.46	$5.97\ (2.63)^{aA}$	$5.90\ (2.62)^{aA}$	5.94
Mean	1.52	1.237		3.76	2.1	
For comparing mean of	S.Em ±	CD (0.05)		S.Em ±	CD (0.05)	
UV exposure time (A)	0.042	0.123		0.082	(0.239)	
L. saksenae and *L. lecanii* (B)	0.021	0.061		0.041	(0.119)	
A x B	0.060	0.173		0.117	(0.337)	

Values sharing same alphabets in superscript are statistically on par based on ANOVA. Upper case alphabets depict statistical significance between fungi and lowercase alphabets depicts the same across the exposure duration. DAI-Days after inoculation. Figures in the parentheses are square root transformed values.

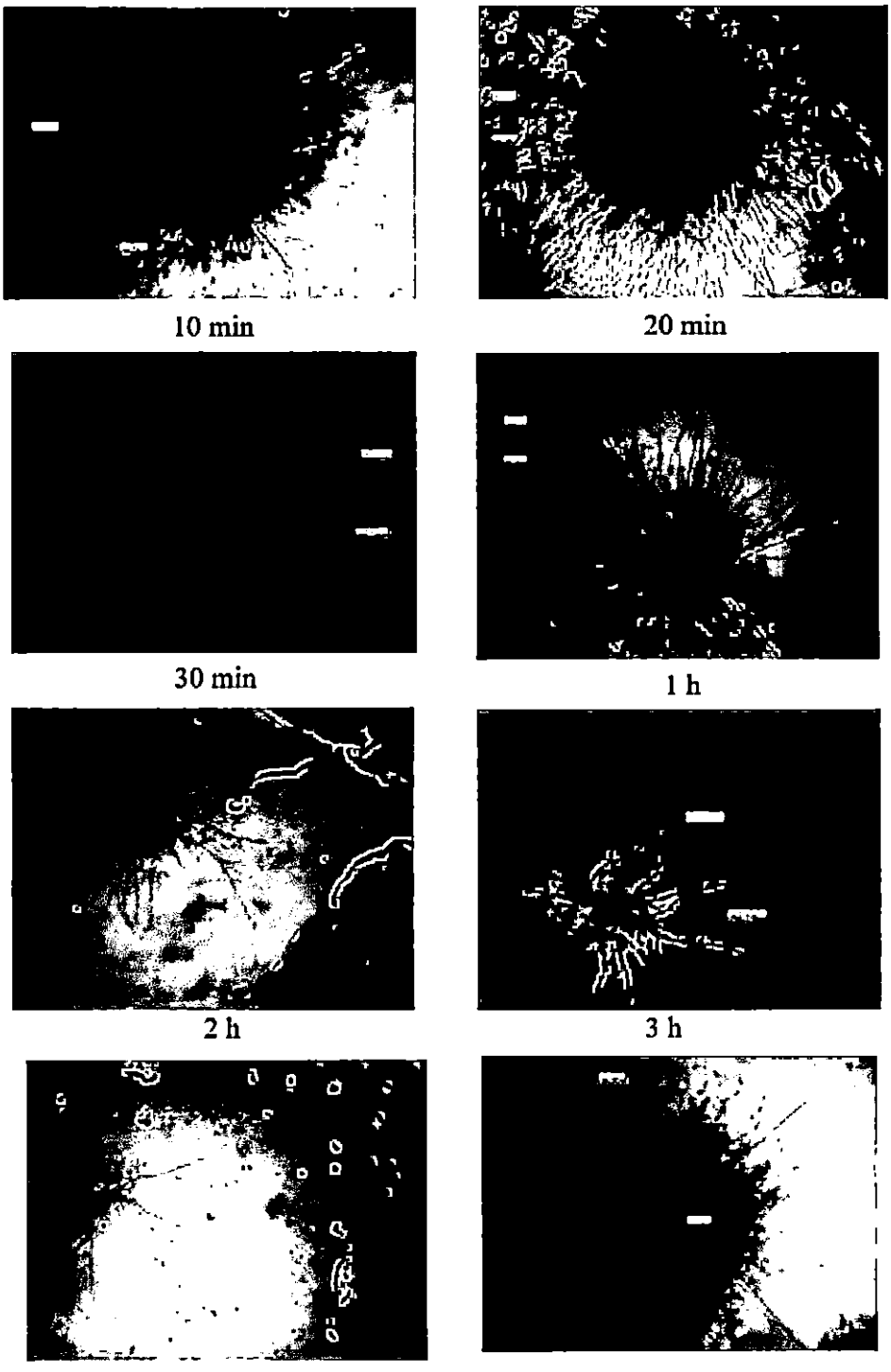


Plate 15. Colony growth of *Lecanicillium saksenae* exposed to UVB (48h, 40X)

4.6.2.1.2 L. lecanii

Growth of *L. lecanii* after 10, 20 and 30 min exposures did not vary significantly, the mean colony diameter being 1.667 mm, 1.63 mm and 1.623 mm respectively (Plate 16). In 1h exposure, the growth was 1.07 mm which was higher to the growth noted in 2 and 3h (0.828 mm and 0.405 mm). Least growth (0.253 mm) was recorded in 4h exposure. The growth observed in unexposed control was 2.418 mm.

4.6.2.2 Effect of UVB on Sporulation

4.6.2.2.1 L. saksenae

Analysis of data revealed that the spore count noted in 10, 20 and 30 min exposure was significantly high among the treatments (5.46 x 10^7 spores mL, 5.23 x 10^7 spores mL^{-1} and 5.15 x 10^7 spores mL^{-1}). At 1, 2, 3 and 4 h of exposure, the spore load was 2.38 x 10^7 mL^{-1}, 2.46 x 10^7 mL^{-1}, 1.8 x 10^7 mL^{-1} and 1.67 x 10^7 mL^{-1} which were statistically on par with each other. Spore count of 5.97 x 10^7 mL^{-1} was recorded in un exposed control.

4.6.2.2.2 L. lecanii

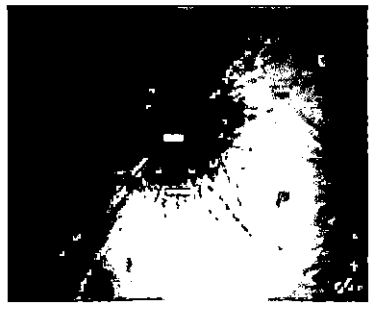
The spore count of *L. lecanii* was significantly affected by UVB. In 10 min exposure, the count was 2.21 x 10^7 spores mL^{-1}. It was 2.13 x 10^7 mL^{-1} and 2.05 x 10^7 mL^{-1} when exposed for 20 min and 30 min, which were on par with each other and significantly high to 1, 2, 3 and 4 h exposures where the spore load was 1.13 x 10^7 mL^{-1}, 1.19 x 10^7 mL^{-1}, 1.1 x 10^7 mL^{-1} and 1.06 x 10^7 mL^{-1} respectively which were similar to each other. In the control, the spore load was 5.9 x 10^7 mL^{-1}.

4.6.2.3 Effect of UVB on Germination

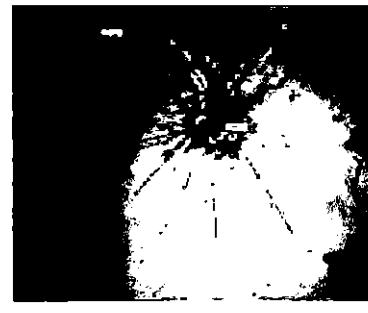
UVB was found to adversely affect the germination capacity of both the fungi (Table 28).

10 min

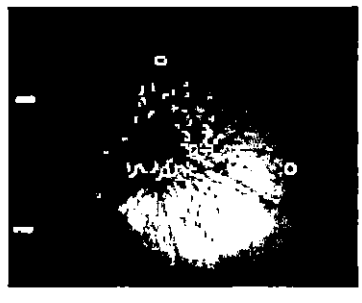
20 min



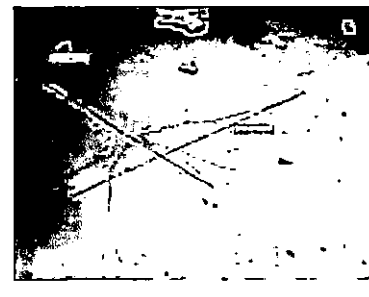
30 min



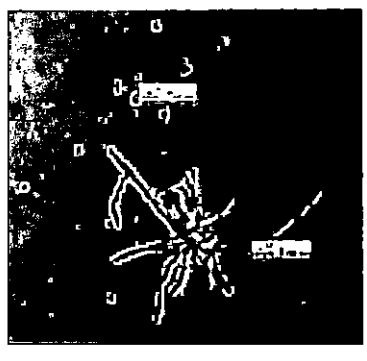
1 h



2 h



3 h



4 h

Control

Plate 16. Colony growth of *Lecanicillium lecanii* exposed to UVB (48h, 40X)

Table 28. Effect of UVB on germination of *Lecanicillium saksenae* and *Lecanicillium lecanii*

Time of exposure	Germination after 24 h. (%)			Germination after 48 h. (%)		
	L. saksenae	*L. lecanii*	Mean	*L. saksenae*	*L. lecanii*	Mean
10 min	29.33 (32.74)^{bB}	41.33 (40.00)^{bA}	35.33	39.33 (38.82)^{bB}	51.67 (45.96)^{bA}	45.50
20 min	23.67 (29.09)^{bB}	33.67 (35.44)^{cA}	28.67	35.67 (36.67)^{bB}	42.67 (40.77)^{cA}	39.17
30 min	15.67 (23.19)^{cB}	27.67 (31.72)^{dA}	21.67	21.00 (27.21)^{cB}	38.33 (38.25)^{cA}	29.67
1h.	9.00 (17.44)^{dA}	9.67 (18.08)^{efA}	9.33	16.00 (23.55)^{cdA}	12.67 (20.81)^{dB}	14.33
2h.	9.33 (17.63)^{dA}	10.67 (19.03)^{efA}	10.00	15.33 (22.97)^{dA}	12.00 (20.19)^{dB}	13.67
3h.	7.67(16.07)^{dA}	7.33 (15.6)^{fA}	7.50	14.00 (21.83)^{dA}	8.67(17.12)^{eB}	11.33
4h.	9.67 (18.05)^{dA}	8.00 (16.41)^{efB}	8.83	13.33 (21.37)^{dA}	9.33 (17.75)^{eB}	11.33
Unexposed (control)	100 (90.00)^{aB}	98.00 (83.44)^{aA}	99.00	100 (90.00)^{aA}	100 (90.00)^{aA}	100
Mean	25.54	29.54		31.83	34.42	
For comparing mean of	S. Em ±	CD (0.05)		S. Em ±	CD (0.05)	
UV exposure time (A)	1.10	(3.17)		0.83	(2.38)	
L. lecanii and L. saksenae (B)	0.55	(1.58)		0.41	(1.19)	
A x B	1.55	(4.48)		1.17	(3.37)	

Figures in the parentheses are angular transformed values for germination. Values sharing same alphabets in superscript are statistically on par based on ANOVA. Upper case alphabets depict statistical significance between fungi and lowercase alphabets depicts the same across the exposure duration.

4.6.2.3.1 L. saksenae

After 24 h, germination rate of 29.33 per cent and 23.67 per cent was recorded at 10 and 20 min exposure respectively which was significantly more compared to 30 min exposure (15.67 per cent). At 1h, 2h, 3h and 4 h exposure, the germination rate was 9.00, 9.33, 7.67 and 9.67 per cent respectively which were in parity with each other. In the control, there was 100 per cent germination.

At the end of 48h, the rate of germination was 39.33 per cent and 35.67 per cent in 10 and 20 min exposure which was followed by the germination rate (21.00 per cent) in 30 min exposure. At 1, 2, 3 and 4 h exposure the rate of germination was 16.00, 15.33, 14.00 and 13.33 per cent, which were on par with each other. In the control, germination rate was 100 per cent.

4.6.2.3.2 L. lecanii

After 24h, the highest rate of germination noted was 41.33 per cent in 10 min exposure, followed by that observed in 20 and 30 min exposure (33.67 and 27.67 per cent), which were significantly different from each other. A very low germination rate of 9.67 per cent, 10.67 per cent and 8.00 per cent was recorded at 1 h, 2h and 4 h of exposure which were on par with each other followed by 7.33 per cent at 3h exposure duration. Spore germination of 98 per cent was observed at unexposed control.

After 48 h, highest germination of 51.67 per cent was recorded in 10 min exposure which was followed by that in 42.67 per cent at 20 min exposure and 38.33 per cent at 30 min exposure which were on par with each other. At 1h, 2h, 3h and 4 h exposures, the rate was 12.67, 12.00, 8.67 and 9.33 per cent respectively which were statistically on par with each other. Hundred per cent germination was observed in the unexposed control.

4.6.2.4 Comparative Tolerance of L. saksenae and L. lecanii to UVB

L. saksenae exhibited higher colony growth than *L. lecanii*, in all the exposures. Colony growth ranged between 2.224 to 2.058 mm at 10, 20 and 30 min exposure duration in *L. saksenae* which was significantly higher than that observed in *L. lecanii* where colony growth ranged between 1.667 to 1.623 mm. At 1, 2, 3 and 4 h exposure, colony growth ranged between 0.363 mm to 1.34 mm in *L. saksenae* whereas it was significantly lower in *L. lecanii* (0.253 to 1.070 mm).

Spore count was significantly more in *L. saksenae* ranging from 1.67 x 10^7 mL^{-1} to 5.46 x 10^7 mL^{-1} while it ranged between 1.06 x 10^7 mL^{-1} to 2.21 x 10^7 mL^{-1} in *L. lecanii* across the exposure duration. In unexposed control no significant difference was observed between *L. saksenae* and *L. lecanii* (5.97 x 10^7 spores mL^{-1} and 5.9 x 10^7 spores mL^{-1} respectively).

At higher exposure duration spore of *L. saksenae* exhibited significantly higher germination compared to *L. lecanii* at 48 h. In 1h, 2h, 3h and 4h exposures, the germination noted was 16.00, 15.33, 14.00 and 13.33 per cent respectively in *L. saksenae* which were significantly higher than that noted in *L. lecanii* (12.67, 12.00, 8.67 and 9.33 per cent respectively).

4.7 INDUCED UV TOLERANCE

Induced UV tolerance was assessed by observing colonies from germinated conidia that were exposed to longer exposure of higher wavelength, UV C (germicidal)

4.7.1 Mutagenesis of *L. saksenae*

Germinated conidia exposed to UV C radiation (germicidal) for 10, 20, 30 minutes and colony at highest exposure duration was selected. At 10 min of exposure many colonies were observed and no colony was observed at 30 min of exposure. At 20 minute of exposure two colony tolerant to UVC was observed. These two colonies were plated to separate plates and further exposed to UV C

radiation for two more generation and finally one colony selected for further studies. Colony was named as LsUVM (*L. saksenae* UV mutant) and mother culture named as WTLs (wild type *L. saksenae*).

4.7.1.1 Phenotypic Changes in L. saksenae UV Mutant

4.7.1.1.1 Performance of UV mutant (LsUVM) in UVA irradiance

Tolerance of *L. saksenae* UV mutant (LsUVM) in comparison with its wild type (WTLs) to UVA irradiation was assessed in terms of phenotypic growth is as below.

In the mutant, the growth recorded at 48 h after inoculation (Table 29) in 30 min exposure was 2.639 mm, while in its wild type the growth was significantly low (2.173 mm). In 2h and 4h exposures too, the growth in mutant and wild varied significantly. It was 2.028 mm and 1.508 mm (2h) and 1.2 mm and 0.643 mm (4h), respectively.

Spore count recorded on 21 DAI, revealed no significant difference between mutant and wild type. In LsUVM, it ranged between 5.24 x 10^7 spores mL^{-1} (4h)$^-$ to 4.1 x 10^7 mL^{-1} (30 min). In WTLs the count ranged between 5.3 x 10^7 spores mL^{-1} (4h) to 4.83 x 10^7 spores mL^{-1} in 30 min.

Germination recorded after 24 h of inoculation (Table 30) revealed that, after 24 h, LsUVM had a significantly high rate of 45.50 per cent while its wild recorded 27.50 per cent in 30 min exposure. In 2h, the rate off germination was 20.25 per cent in the mutant while it was 10.75 per cent in wild. In 4h, germination of 14.5 per cent was observed in mutant while it was 8.25 per cent in wild type. No significant difference was observed in unexposed control.

4.7. 1.1.2 Comparative Tolerance of LsUVM and WTLs to UVA

At 30 min exposure, higher colony growth (2.639 mm) was observed in LsUVM which was significantly more than observed in WTLs (2.173 mm). In 2h exposure, the mean colony diameter was 2.028 mm in LsUVM which was significantly more than that in WTLs (1.508 mm).

Table 29. Effect of UVA on growth and sporulation of *Lecanicillium saksenae* UV mutant

Exposure time	Colony growth (mm) ± S.E at 48 h			Spore count (x10 ^{7} spores mL^{1}) 21 DAI		
	Ls UVM	WT Ls	Mean	Ls UVM	WT Ls	Mean
30 min	2.64 ± 0.303^{aA}	2.17± 0.093^{bB}	2.406	4.10 (2.25)	4.83 (2.41)	4.46
2h	2.02±0.076^{bA}	1.50 ± 0.023^{cB}	1.768	4.52 (2.35)	5.29 (2.48)	4.91
4h	1.20 ± 0.066^{cA}	0.64 ± 0.0121^{dB}	0.925	5.24 (2.49)	5.3 (2.51)	5.27
Unexposed (control)	2.68 ± 0.037^{aA}	2.65 ± 0.025^{aB}	2.668	4.78 (2.4)	6.19 (2.67)	5.48
Mean	2.138	1.745		4.66 (2.37)	5.40 (2.52)	
For comparing mean of	S. Em ±	CD (0.05)		S. Em ±	CD (0.05)	
UV exposure time (A)	0.033	(0.096)		0.083	NS	
Ls UVM and WT Ls (B)	0.023	(0.068)		0.059	NS	
A x B	0.046	(0.136)		0.117	NS	

LsUVM - *L. saksenae* UV mutant; WTLs - Wild type *L. saksenae* (non-mutant); Upper case alphabets depict statistical significance between mutants and lowercase alphabets depicts the same across the exposure duration. Figures in the parentheses are square root transformed values.

Table 30. Effect of UVA on germination of *Lecanicillium saksenae* UV mutant

Exposure time	Germination after 24 h (%)			Germination after 48 h (%)		
	Ls UVM	WT Ls	Mean	Ls UVM	WT Ls	Mean
30 min	45.50 (42.42)^{bA}	27.5 (31.62)^{bB}	36.50	63.00 (52.55)^{bA}	39.00 (38.65)^{bB}	51.00
2h	20.25 (26.73)^{cA}	10.75 (19.12)^{cB}	15.50	34.5 (35.94)^{cA}	11.75 (19.98)^{cB}	23.13
4h	14.5 (22.27)^{dA}	8.25 (16.55)^{dB}	11.38	20.75 (27.06)^{dA}	10.25 (18.6)^{cB}	15.50
Control	99.00 (87.12)^{aA}	98.50 (85.08)^{aB}	98.75	100 (90)^{aA}	100 (90)^{aA}	100
Mean	44.81 (44.63)	36.25 (38.09)		54.56 (51.39)	40.25 (41.81)	
For comparing mean of	S. Em ±	CD (0.05)		S. Em ±	CD (0.05)	
UV exposure time (A)	1.183	(3.472)		0.653	(1.935)	
Ls UVM and WT Ls (B)	0.836	(2.455)		0.462	(1.368)	
A x B	1.672	N/A		0.924	(2.736)	

Ls UVM - *L. saksenae* UV mutant; WT Ls - Wild type *L. saksenae*. Figures in the parentheses are arc sine transformed value. Upper case alphabets depict statistical significance between mutants and lower-case alphabets depicts the same across the exposure duration

Similar significant difference at 4h of exposure was observed between growth of LsUVM (1.206 mm) and WTLs (0.643 mm).

The germination rate at 48 h exposure, 30 min exposure to UVA, the germination rate in LsUVM was significantly high (63 per cent) to its wild type (39 per cent). Germination of 34.5 per cent and 20.75 per cent was recorded in LsUVM at 2h and 4h exposure which was significantly higher than the corresponding values in WTLs where germination observed was 11.75 per cent and 10.75 per cent respectively.

4.7. 1.1.3 Performance of UV mutant (LsUVM) in UVB irradiance

Tolerance of *L. saksenae* UV mutant (LsUVM) in comparison with its Wild type (WTLs) to UVB irradiation was assessed in terms of phenotypic growth is as below.

In the mutant, the growth recorded at 48 h after inoculation (Table 31) in 30 min exposure was 2.341 mm, while in its wild type the growth was significantly low (2.038 mm). In 2h and 4h exposures too, the growth in mutant and wild varied significantly. It was 1.081 mm and 0.818 mm (2h) and 0.555 mm and 0.291 mm (4h), respectively.

Spore count recorded on 21 DAI, revealed no significant difference between mutant and wild type. In LsUVM, it ranged between 4.92 x 10^7 spores mL^{-1} (4h) to 4.02 x 10^7 mL^{-1} (30 min). In WTLs the count ranged between 4.19 x 10^7 spores mL^{-1} (4h) to 3.24 x 10^7 spores mL^{-1} in 2h.

Germination recorded after 24 h of inoculation (Table 32) revealed that, after 24 h, LsUVM had a significantly high rate of 45.25 per cent while its wild recorded 39 per cent in 30 min exposure. In 2h, the rate off germination was 18.5 per cent in the mutant while it was 11.70 per cent in wild. In 4h, germination of 17.5 per cent was observed in mutant while it was 10.25 per cent in wild type. No significant difference was observed in unexposed control.

Table 31. Effect of UVB on growth and sporulation of *Lecanicillium saksenae* UV mutant

Exposure time	Colony growth (mm) at 48 h ± SE			Spore count (10^7 spores mL^{-1}) 21 DAI		
	Ls UVM	WT Ls	Mean	Ls UVM	WT Ls	Mean
30 min	2.34 ± 0.046^{bA}	2.03 ± 0.035^{bB}	2.19	4.02 (2.23)	3.92 (2.18)	3.97
2h	1.08 ± 0.031^{cA}	0.81 ± 0.0601^{cB}	0.95	3.70 (2.16)	3.24 (2.03)	3.47
4h	0.55 ± 0.0172^{dA}	0.29 ± 0.026^{dB}	0.423	3.94 (2.21)	3.89 (2.2)	3.92
Unexposed (control)	2.70 ± 0.0545^{aA}	2.69 ± 0.026^{aA}	2.699	4.92 (2.41)	4.19 (2.27)	4.56
Mean	1.671	1.459		4.14 (2.25)	3.81 (2.17)	
For comparing mean of	S. Em ±	CD (0.05)		S. Em ±	CD (0.05)	
UV exposure time (A)	0.028	(0.083)		0.12	NS	
Ls UV and WT Ls (B)	0.02	(0.058)		0.085	NS	
A x B	0.04	(0.117)		0.17	NS	

Ls UVM - *L. saksenae* UV mutant; WT Ls - Wild type *L. saksenae* Upper case alphabets depicts statistical significance between mutants and lowercase alphabets depicts the same across the exposure duration.
Figures in the parentheses are square root transformed values

Table 32. Effect of UVB on germination percentage of *Lecanicillium saksenae* UV mutant

Exposure time	Germination after 24 h (%) at 48 h			Germination after 48 h (%)		
	Ls UVM	WT Ls	Mean	Ls UVM	WT Ls	Mean
30 min	34.00 (35.66)^{bA}	27.00 (31.29)^{bB}	30.50 (33.48)	45.25 (42.27)^{aA}	39.00 (38.65)^{bB}	42.13 (40.46)
2h	15.25 (22.98)^{cA}	10.25 (18.64)^{cB}	12.75 (20.81)	18.5 (25.44^{bA}	11.70 (19.98)^{cB}	15.13 (22.71)
4h	12.00 (20.16)^{dA}	8.25 (16.67)^{dB}	10.13 (18.41)	17.5 (24.68)^{bA}	10.25 (18.6)^{cB}	13.88 (21.64)
Unexposed (control)	99 (87.12)^{aA}	98.5 (85.08)^{aA}	98.75 (86.1)	100 (90.00)^{aA}	100 (90.00)^{aA}	
Mean	40.06 (41.48)	36.00 (37.92)		54.56 (51.39)	40.25 (41.81)	
For comparing mean of	S. Em ±	CD (0.05)		S. Em ±	CD (0.05)	
UV exposure time (A)	1.133	(3.327)		0.541	(1.587)	
Ls UVM and WT Ls (B)	0.801	(2.353)		0.382	(1.122)	
A x B	1.603	NS		0.764	(2.245)	

Ls UVM - *L. saksenae* UV mutant; WT Ls -Wild type *L. saksenae* Figures in the parentheses are arc sine transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA. Upper case alphabets depicts statistical significance between mutants and lower case alphabets depicts the same across the exposure duration

4.7. 1.1.4 Comparative Tolerance LsUVM and WTLs to UVB

At 30 min exposure, higher colony growth (2.341 mm) was observed in LsUVM which was significantly more than observed in WTLs (2.038 mm). In 2h exposure, the mean colony diameter was 1.081 mm in LsUVM which was significantly more than that in WTLs (0.818 mm). Similar significant difference at 4h of exposure was observed between growth of LsUVM (0.55 mm) and WTLs (0.291 mm).

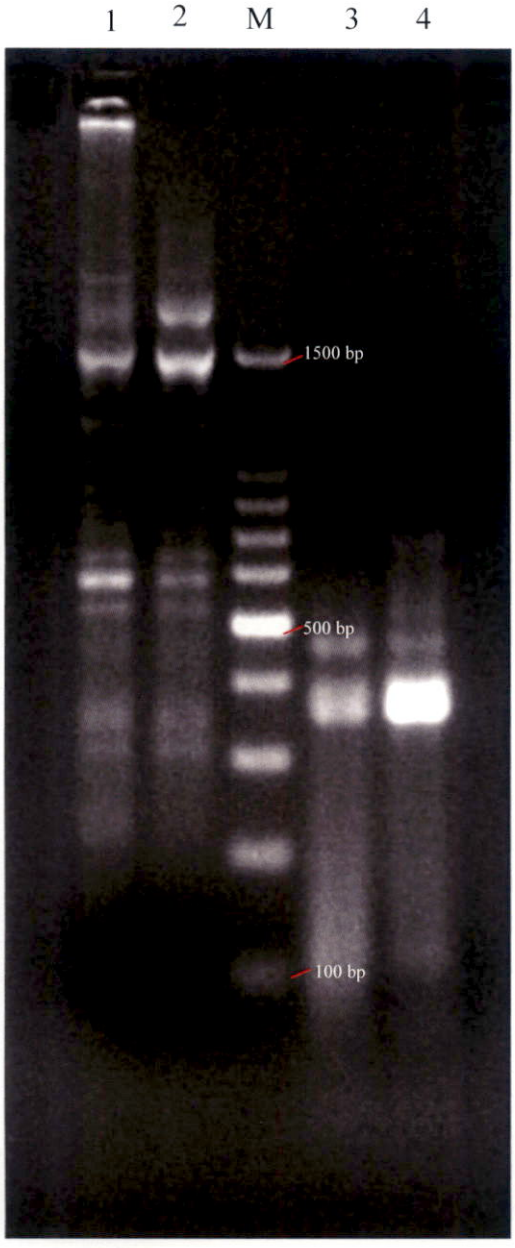
The germination rate at 48 h after treatment reveal that 30 min exposure to UVA exposure, the germination rate was significantly high (45.25 per cent) compared to its wild type (39.00 per cent). Germination of 18.5 per cent and 11.70 per cent was recorded in treatment 2h and 4h exposures which were significantly higher than the corresponding values in WTLs (17.5 per cent and 10.25 per cent respectively).

4.7.1.2 Molecular Changes in L. saksenae UV Mutant

The REP band profiles obtained by amplifying the DNA isolated from UV mutant and its wild type using REP primer REP1R and REP2I is presented below.

4.7.1.2.1 REP-PCR Profiles of L. saksenae UV Mutant

PCR results of the wild type *L. saksenae* (WTLs) and UV tolerant mutant (LsUVM) is presented in Plate 17. Comparison of binary data using Jaccard's coefficient revealed that UV mutant (LsUVM) was 32 per cent dissimilar to wild type of *L. saksenae* (Table 33 and Fig. 2), indicating the distinct nature of the mutant.



REP1R

Lane 1- Wild type *L.saksenae*

Lane 2- *L.saksenae* UV mutant

Lane M- 100bp ladder

REP2I

Lane 3- Wild type *L.saksenae*

Lane 4- *L.saksenae* UV mutant

Plate 17. Gel profile of UV tolerant *Lecanicillium saksenae* generated with REP Primer

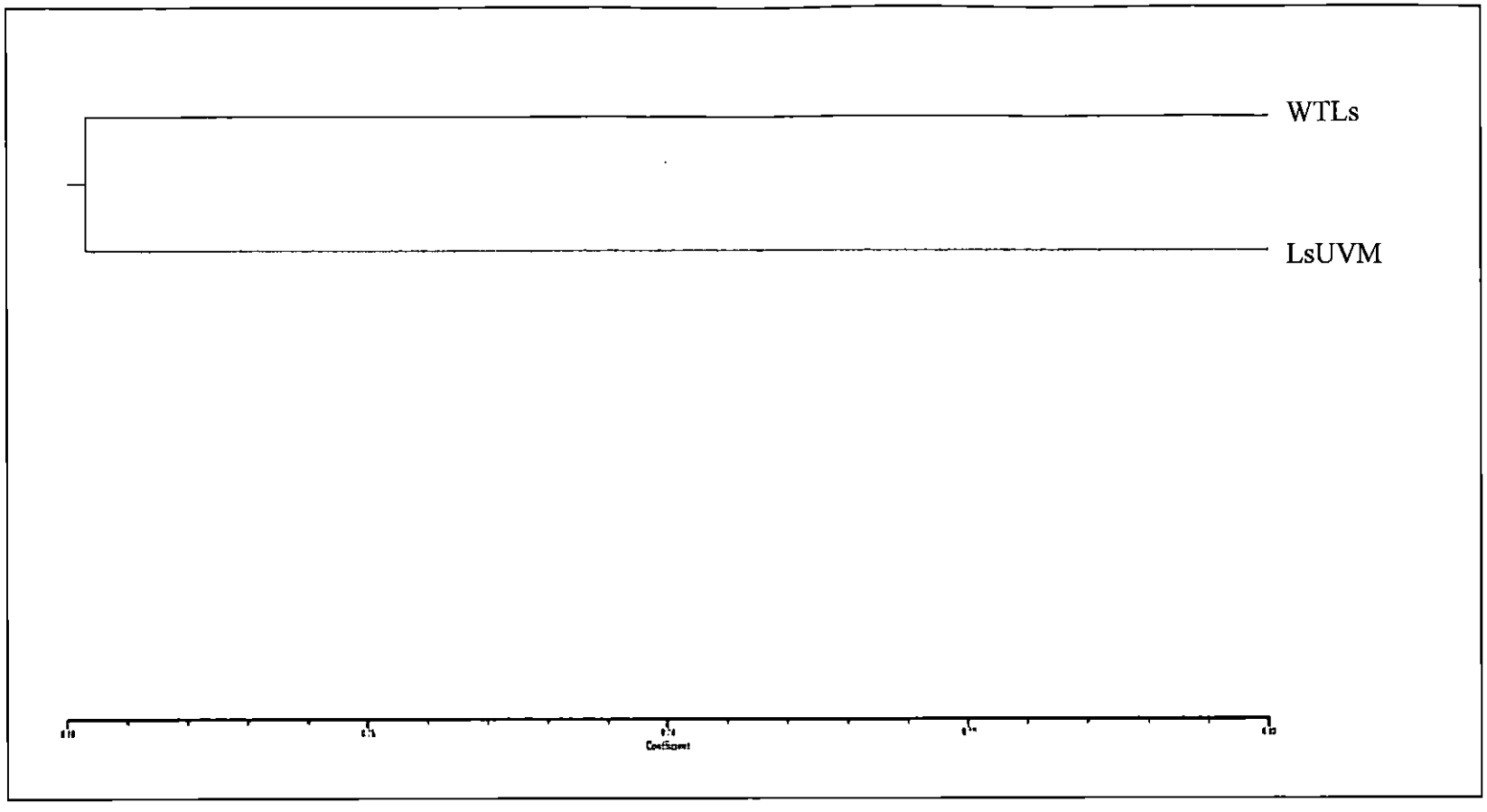


Fig. 2. Dendrogram from UPGMA cluster analysis of DNA banding profiles of *Lecanicillium saksenae* UV tolerant culture, generated with REP Primer . Similarity between the profiles was estimated with Jaccard's correlation coefficient (WTLS- wild type *L.saksenae,* LsUVM - *L.saksenae* UV Mutant).

Table 33. Similarity matrix of wild type *Lecanicillium saksenae* and its UV mutant based on Jaccard's coefficient

	Wild type *L. saksenae* (non-mutant)	LsUVM- *L. saksenae* UV mutant
Wild type *L. saksenae*	1.00	
LsUVM- *L. saksenae* UV mutant	0.68	1.00

4.7.2 Mutagenesis of L. lecanii

At 10 min of exposure to UVC many colony forming units were observed and in 20 and 30 min one each colony was observed. These two colonies were plated to separate plates and further exposed to UVC radiation for two more generation and finally two colony were selected for further studies. Colony was named LlUV1 (mutant at 30 min) and LlUV2 (mutant at 20 min) and mother culture was named as WTLl (Wild type *Lecanicillium lecanii).*

4.7.2.1 Phenotypic Changes in L. lecanii UV Mutant

4.7.2.1.1 Performance of UV mutant of L. lecanii in UVA Irradiance

Tolerance of *L. lecanii* UV mutants in comparison with its Wild type to UVA irradiation was assessed in terms of phenotypic growth is depicted in Table 34.

Colony growth of LlUV1, LlUV2 and WTLl was significantly affected during all the exposure duration. At 30 min, growth of 2.44 mm, 1.71 and 1.58 mm was recorded in LlUV1, WTLl and LlUV2 respectively. At 2h and 4 h exposure growth of 1.94 mm and 1.74 mm respectively observed was in LlUV1 while corresponding growth was 1.14 mm and 0.43 mm in LlUV2. In WTLl growth was 1.13 mm (at 2h) and 0.43 mm (at 4h) exposure respectively. In unexposed control growth was 2.44 mm (LlUV1), 2.25mm (WTLl) and 2.19 mm (LlUV2).

Table 34. Effect of UVA on growth and sporulation of *Lecanicillium lecanii* UV mutant

Exposure time	Colony growth (mm) ± S.E at 48 h.				Spore count (10^7 spores mL^{-1}) 21DAI			
	LlUV1	LlUV2	WT Ll	Mean	LlUV1	LlUV2	WTLl	Mean
30 min	2.44 ± 0.14^{aA}	1.58 ± 0.04^{bC}	1.71 ± 0.02^{bB}	1.909	2.30 (1.8)	2.23 (1.77)	1.80 (1.66)	2.11 (1.74)
2h	1.94 ± 0.04^{bA}	1.14 ± 0.05^{cB}	1.13 ± 0.02^{cB}	1.401	3.60 (2.13)	2.20 (1.76)	3.04 (1.97)	2.95 (1.95)
4h	1.74 ± 0.05^{cA}	0.43 ± 0.07^{dB}	0.43 ± 0.02^{dB}	0.87	3.83 (2.18)	2.80 (1.9)	3.97 (2.2)	4.20 (2.25)
Unexposed control	2.44 ± 0.16^{aA}	2.19 ± 0.09^{aB}	2.25 ± 0.03^{aB}	2.291	3.73 (2.16)	2.93 (1.93)	3.40 (2.07)	3.35 (2.05)
Mean	2.14	1.336	1.379		3.36	3.04	3.05	
For comparing mean of	S. Em ±	CD (0.05)			S. Em ±	CD (0.05)		
UV exposure time (A)	0.033	(0.096)			0.108	NS		
Ll UV1, Ll UV2 and WT Ll (B)	0.029	(0.083)			0.093	NS		
A x B	0.058	(0.166)			0.187	NS		

Ll UV1- *L. lecanii* UV mutant one ; Ll UV2- *L. lecanii* UV mutant two; WT Ll – Wild type *L. lecanii*. Upper case alphabets depict statistical significance between mutants and lowercase alphabets depicts the same across the exposure duration

Spore count recorded on 21 DAI, revealed no significant difference between mutants and wild type. In LlUV1, it ranged between 3.83 x 10^7 spores mL^{-1} (4h) to 2.3 x 10^7 mL^{-1} (30 min) and In LlUV2 the count ranged between 2.8 x 10^7 spores mL^{-1} (3h) to 2.2 x 10^7 spores mL^{-1} (2h). In WTLl the count ranged between 5.3 x 10^7 spores mL^{-1} (4h) to 4.83 x 10^7 spores mL^{-1} in 30 min.

Germination recorded after 24 h reveal that, all the cultures were significantly inhibited across the exposure duration (Table 35). At 30 min exposure germination of 39.25, 18.25 and 17.5 per cent was observed in LlUV1, LlUV2 and WTLl respectively. At 2h and 4h of exposure LlUV1 exhibited 21.75 and 16.25 per cent germination which was significantly more than LlUV2 (11.75 and 8.5 per cent) and WTLl (10.75 and 9 per cent) respectively. No significant difference was found in unexposed control.

4.7.2.1.2 Comparative tolerance of LlUV1, LlUV2 and WTLl to UVA Irradiance

At all the exposure duration highest colony growth was observed in LlUV1 with growth of 2.44 mm, 1.94 mm and 1.74 mm at 30 min, 2h and 4h exposure respectively. There was no significant difference observed between LlUV2 and WTLl in all the exposure duration.

Germination rate at 48 h, highest germination per cent was observed in LlUV1 in all the exposure duration. At 30 min, 2h and 4h of exposure germination of 59.5, 29 and 27 per cent was recorded respectively which were statistically significant from LlUV2 and WTLl. No significant difference was observed between LlUV2 and WTLl.

4.7.2.1.3 Performance of UV mutant of L. lecanii in UVB Irradiance

Tolerance of *L. lecanii* UV mutants in comparison with its Wild type to UVB irradiation was assessed in terms of phenotypic growth is depicted in Table 36.

Colony growth of LlUV1, LlUV2 and WTLl was significantly affected during all the exposure duration.

Table 35. Effect of UVA on germination of *Lecanicillium lecanii* UV mutants

Exposure time	Germination after 24 h (%)				Germination after 48 h (%)			
	Ll UV1	Ll UV2	WT Ll	Mean	Ll UV1	Ll UV2	WT Ll	Mean
30 min	39.25 (38.76)^{bA}	17.5 (24.68)^{bB}	18.25 (25.24)^{bB}	25.00	59.50 (50.49)^{aA}	24.00 (29.33)^{aB}	24.5 (29.61)^{aB}	36.00
2h	21.75 (27.73)^{cA}	11.75 (19.87)^{cB}	10.75 (19.08)^{cB}	14.75	29.00 (32.48)^{bA}	15.50 (23.11)^{bB}	16.25 (23.69)^{bB}	20.08
4h	16.25 (23.76)^{dA}	8.50 (16.88)^{dB}	9.00 (17.42)^{cB}	11.25	27.00 (31.05)^{bA}	14.50 (22.33)^{bB}	15.75 (23.38)^{bB}	19.25
Unexposed control	98.25 (84.62)^{aA}	97.25 (83.4)^{aA}	97.75 (82.71)^{aA}	97.75	100 (90.00)^{cA}	100 (90.00)^{cA}	100 (90.00)^{cA}	100
Mean	43.88	33.75	33.94		53.88	38.5	39.13	
For comparing mean of	S. Em ±	CD (0.05)			S. Em ±	CD (0.05)		
UV exposure time (A)	1.133	(3.263)			0.763	2.198		
Ll UV1, Ll UV2 and WT L1 (B)	0.981	(2.826)			0.661	1.904		
A x B	1.962	NS			1.322	3.807		

LUV1- *L. lecanii* UV mutant one; Ll UV2- *L. lecanii* UV mutant two; WT Ll– Wild type *L. lecanii*. Figures in the parentheses are arc sine transformed values. Upper case alphabets depict statistical significance between mutants and lower case alphabets depict the same across the exposure duration

Table 36. Effect of UVB on growth and sporulation of *Lecanicillium lecanii* UV mutant

Exposure time	Colony growth (mm) ± S.E at 48 h				Spore count (10^7 spores mL^{-1}) 21 DAI			
	Ll UV1	Ll UV2	WT Ll	Mean	Ll UV1	Ll UV2	WT Ll	Mean
30 min	2.11 ± 0.12^{bA}	1.47 ± 0.07^{bB}	1.55 ± 0.09^{bB}	1.711	2.25 (1.79)	2.01 (1.72)	1.58 (1.60)	1.94
2h	1.40 ± 0.14^{cA}	0.96 ± 0.09^{cB}	0.94 ± 0.07^{cB}	1.098	3.10 (2.00)	1.70 (1.62)	2.54 (1.84)	2.45
4h	0.87 ± 0.06^{dA}	0.38 ± 0.07^{dB}	0.40 ± 0.08^{dB}	0.549	3.33 (2.06)	4.30 (2.27)	3.47 (2.08)	3.70
Unexposed control	2.44 ± 0.11^{aA}	2.19 ± 0.07^{aB}	2.25 ± 0.03^{aB}	2.291	3.23 (2.03)	2.43 (1.79)	2.9 (1.93)	2.85
Mean	1.705	1.248	1.284		2.98	2.61	2.62	
For comparing mean of	S. Em ±	CD (0.05)			S. Em ±	CD (0.05)		
UV exposure time (A)	0.047	(0.136)			0.113	NS		
Ll UV1, Ll UV2 and WT Ll (B)	0.041	(0.118)			0.098	NS		
A x B	0.082	NS			0.196	NS		

LlUV1- *L. lecanii* UV mutant one ; Ll UV2- *L. lecanii* UV mutant two; WTLl – Wild type *L. lecanii.* Upper case alphabets depict statistical significance between mutants and lowercase alphabets depicts the same across the exposure duration

At 30 min, growth of 2.11 mm, 1.55 and 1.47 mm was recorded in LlUV1, WTLl and LlUV2 respectively. At 2h and 4h exposure growth of 1.40 mm and 0.87 mm respectively observed was in LlUV1 while corresponding growth was 0.96 mm and 0.38 mm in LlUV2. In WTLl growth was 0.94 mm (at 2h) and 0.4 mm (at 4h) exposure respectively. In unexposed control growth was 2.44 mm (LlUV1), 2.25mm (WTLl) and 2.19 mm (LlUV2).

Spore count recorded on 21 DAI, revealed no significant difference between mutants and wild type. In LlUV1, it ranged between 2.25 x 10^7 spores mL^{-1} (30min) to 3.33 x 10^7 mL^{-1} (4h) and in LlUV2 the count ranged between 2.01 x 10^7 spores mL^{-1} (1.72) to 4.3 x 10^7 spores mL^{-1} (2h). In WTLl the count ranged between 1.58 x 10^7 spores mL^{-1} (4h) to 2.9 x 10^7 spores mL^{-1} in 30 min.

Germination recorded after 24 h reveal that, all the cultures were significantly inhibited across the exposure duration (Table 37). At 30 min exposure germination of 27.5, 17.00 and 15.25 per cent was observed in LlUV1, LlUV2 and WTLl respectively. At 2h and 4h of exposure LlUV1 exhibited 20.25 and 18.25 per cent germination which was significantly more than LlUV2 (9.25 and 9.0 per cent) and WTLl (8.75 and 10 per cent) respectively. No significant difference was found in unexposed control.

4.7.2.1.4 Comparative tolerance of LlUV1, LlUV2 and WTLl to UVB irradiance

At all the exposure duration of UVB highest colony growth was observed in LlUV1 with growth of 2.11 mm, 1.4 mm and 0.87 mm at 30 min, 2h and 4 h exposure respectively. There was no significant difference observed between LlUV2 and WTLl in all the exposure duration.

Germination data recorded at 48h reveal that highest germination per cent was observed in LlUV1 in all the exposure duration. At 30 min, 2h and 4h of exposure germination of 40.0, 24.75 and 19.75 per cent was recorded respectively which were significantly superior from LlUV2 and WTLl at 48 h of observation.

Table 37. Effect of UVB on germination of *Lecanicillium lecanii* UV mutants

Exposure time	Germination after 24 h (%)				Germination after 48 h (%)			
	Ll UV1	Ll UV2	WT Ll	Mean	LlUV1	LlUV2	WTLL	Mean
30 min	27.50 (31.59)^{bA}	17.00 (24.28)^{bB}	15.25 (22.9)^{bB}	19.92	40.00 (39.21)^{bA}	20.00 (26.47)^{bB}	20.00 (26.49)^{bB}	26.67
2h	20.25 (26.66)^{cA}	9.25 (17.63)^{cB}	8.75 (17.06)^{cB}	12.67	24.75 (29.83)^{cA}	17.50 (24.72)^{cB}	15.00 (22.72)^{cB}	19.08
4h	18.25 (25.27)^{cA}	9.00 (17.38)^{cB}	10.00 (18.38)^{cB}	12.50	19.75 (26.37)^{dA}	10.50 (18.86)^{dB}	11.75 (20.00)^{cB}	14.00
Unexposed control	98.25 (84.62)^{aA}	97.25 (83.40)^{aA}	98.75 (86.77)^{aA}	98.08	98.25 (84.62)^{aB}	97.25 (83.4)^{aB}	100 (90)^{aA}	98.50
Mean	41.06	33.13	33.19		45.69	36.31	36.69	
For comparing mean of	S. Em ±	CD (0.05)			S. Em ±	CD (0.05)		
UV exposure time (A)	1.161	3.343			1.008	2.904		
LlUV1, LlUV2 and WTLl (B)	1.005	2.895			0.873	2.515		
A x B	2.01	NS			1.747	5.03		

Ll UV1- *L. lecanii* UV mutant one ; Ll UV2- *L. lecanii* UV mutant two; WT Ll - Wild type *L. lecanii*. Upper case alphabets depict statisti significance between mutants and lowercase alphabets depicts the same across the exposure duration. Values in parenthesis are arc transformed

No significant difference was observed between LIUV2 and WTLl in terms of germination in unexposed control.

4.7.2.2 Molecular Changes in L. lecanii UV mutant

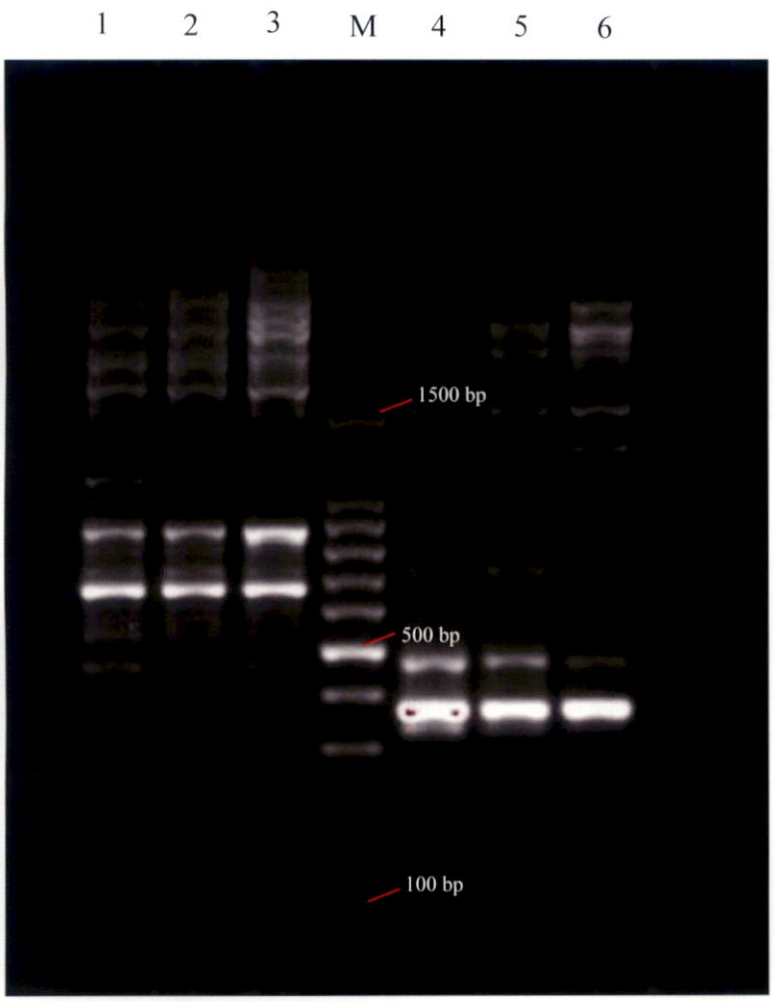
The REP band profiles obtained by amplifying the DNA isolated from UV mutant and its wild type using REP primer REP1R and REP2I is presented below.

4.7.2.2.1 REP-PCR Profiles of L. lecanii UV mutant

The genomic changes of the two UV mutants (LLUV 1 and LIUV2) with respect to their wild type (WTLl), is presented in Plate 18. Cluster analysis of binary data allowed the definition of two major groups (A and B) at similarity of around 66 per cent (Fig. 3). Cluster A divided in two subclusters, where subcluster I had similarity of 75 per cent with subcluster II. Based on Jaccard's coefficient (Table 38), LlUV1 was 31.5 per cent dissimilar to WTLl while the dissimilarity of LlUV2 was 25 per cent. Mutant LlUV1 was 37.5 per cent dissimilar with LlUV2.

Table 38. Similarity matrix of wild type *Lecanicillium lecanii* and its UV mutant based on Jaccard's coefficient

	WTLl - Wild type *L. lecanii*	LlUV2- *L. lecanii* UV mutant two	Ll UV1- *L. lecanii* UV mutant one
WTLl - Wild type *L. lecanii*	1.00		
Ll UV2- *L. lecanii* UV mutant two	0.75	1.00	
Ll UV1- *L. lecanii* UV mutant one	0.6875	0.625	1.00



REP1R

Lane 1- Wild type *L. lecanii*,

Lane 2- LlUV2 – UV mutant

Lane 3- LlUV1 – UV mutant

Lane M- 100bp ladder

REP2I

Lane 6- Wild type *L. lecanii*

Lane 7- LlUV2 - UV mutant

Lane 8- LlUV1- UV mutant

Plate 18. Gel profile of UV tolerant *Lecanicillium lecanii* generated with REP Primer

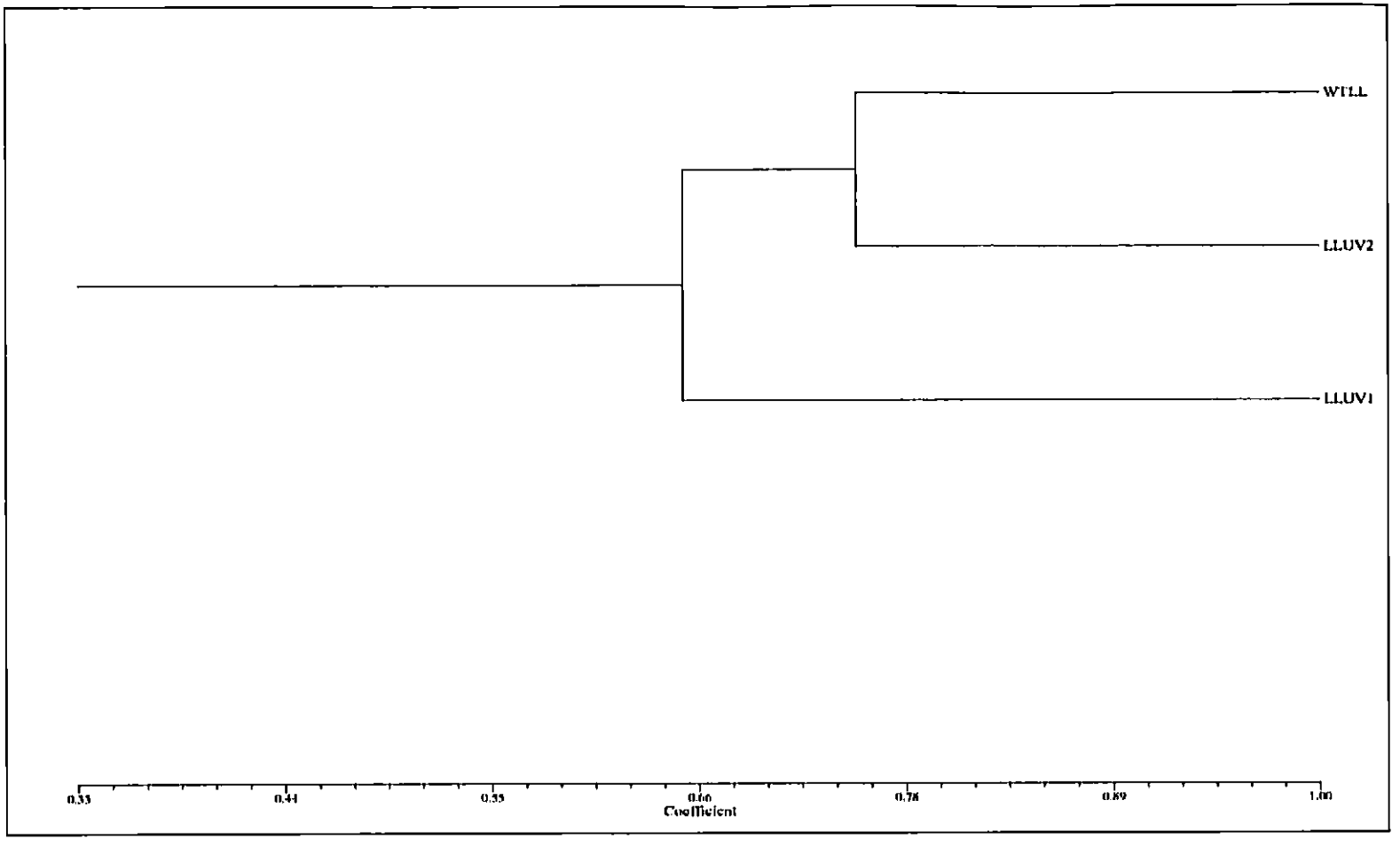


Fig. 3. Dendrogram from UPGMA cluster analysis of DNA banding profiles of *Lecanicillium lecanii* UV tolerant culture, generated with REP Primer . Similarity between the profiles was estimated with Jaccard's correlation coefficient. (WTLl- wild type *L. lecanii,* LlUV1 - *L. lecanii* UV mutant 1, LlUV2- *L. lecanii* UV mutant 2)

4.8 PATHOGENICITY ASSAY OF SELECTED LINES OF *L. saksenae* AND *L. Lecanii*

Pathogenicity of selected lines of *L. saksenae* and *L. lecanii* was tested against the adults of *Leptocorisa oratorius* Fab. and nymphs of *Coccidohystrix insolita* Green respectively.

4.8.1 Pathogenicity *L. saksenae* lines to *L. oratorius*

Pathogenicity of the carbendazim tolerant, hexaconazole tolerant and UV tolerant cultures of *L. saksenae* carried out for selected and non-selected cultures (10^7 spores mL $^{-1}$) alone and also along with respective fungicides is detailed here under (Table 39 and Table 40).

4.8.1.1 Carbendazim Tolerant Culture

On first day of treatment (DAT), highest mortality observed was 60 per cent, in adults sprayed with non-selected *L. saksenae* which was on par with that noted in selected culture (53.33 per cent). Bugs treated with selected + carbendazim 50% WP 0.2% exhibited 50 per cent mortality while no mortality was observed in non-selected + carbendazim 50% WP 0.2 %.

On the third day, 93.33 per cent mortality was observed in non-selected culture which significantly varied from all other treatments. There was 83.33 per cent mortality in the selected culture followed by 73.33 per cent in selected + carbendazim 50% WP 0.2 %.

In the bugs treated with selected + carbendazim 50% WP 0.2 %, mortality of 73.33 per cent was observed and the least mortality (30 per cent) was observed in non-selected + carbendazim 50% WP 0.2 %. No mortality was observed in carbendazim 50% WP 0.2% and control (water).

Table 39. Pathogenicity of carbendazim tolerant strain of *Lecanicillium saksenae* to *Leptocorisa oratorius*

Treatments	Mortality at 24 h. interval (%)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Non selected	60.00 (50.85)[a]	80.00 (63.93)[a]	93.33 (77.62)[a]	100 (89.71)[a]	100 (89.71)[a]
Selected	53.33 (47.01)[a]	66.67 (55.08)[ab]	83.33 (69.98)[ab]	100 (89.71)[a]	100 (89.71)[a]
Non selected + carbendazim 50 % WP 0.2 %	0 (0.29)[b]	0 (0.29)[c]	30.00 (33.00)[c]	36.67 (37.23)[c]	36.67 (37.23)[c]
Selected + carbendazim 50 % WP 0.2 %	53.33 (46.92)[a]	63.33 (52.78)[b]	73.33 (59)[b]	73.33 (59.00)[b]	86.67 (72.19)[b]
Carbendazim 50 % WP 0.2 %	0 (0.29)[c]	0 (0.29)[c]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]
Untreated control	0 (0.29)[c]	0 (0.29)[c]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]
CD (0.05)	8.151	9.014	15.709	3.760	11.305
S.Em ±	12.462	15.817	17.192	18.891	19.520

Figures in the parentheses are arc sine transformed. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Table 40. Pathogenicity of hexaconazole tolerant strain of *Lecanicillium saksenae* against *Leptocorisa oratorius*

Treatments	Mortality at 24 h interval (%)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Non selected	60.00 (50.85)[a]	83.33 (66.15)[a]	100 (89.71)[a]	100 (89.71)[a]	100 (89.71)[a]
Selected	3.33 (6.34)[b]	30.00 (33)[b]	36.67 (36.93)[b]	56.67 (48.93)[b]	66.67 (54.78)[b]
Non-selected + hexaconazole 5 % EC	0 (0.29)[c]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]
Selected + hexaconazole 5% EC 0.15 %	0 (0.29)[c]	6.67 (12.39)[c]	20.00 (26.57)[c]	36.67 (37.23)[c]	53.33 (46.92)[c]
Hexaconazole 5% EC 0.15 %	0 (0.29)[c]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]
Control (water)	0 (0.29)[c]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]	0 (0.29)[d]
CD (0.05)	8.73	9.52	6.849	5.551	3.479
S.Em ±	9.90	13.526	15.972	16.659	17.43

Figures in the parentheses are arc root transformed values. Values sharing same alphabets in superscript are statistically on par based on ANOVA.

On the fifth day, 100 per cent mortality was observed in non-selected culture and selected culture. Selected + carbendazim 50% WP 0.2% caused mortality of 86.67 per cent and least mortality was observed in non-selected + carbendazim 50% WP 0.2%. Untreated insects and insects treated with only carbendazim 50% WP 0.2% no mortality was observed.

Selected and relaxed culture when sprayed alone exhibited same effectiveness as of non-selected culture. Relaxed and selected culture treated along with carbendazim 50% WP exhibited higher mortality compared to non-selected culture + carbendazim 50% WP which clearly proved the induced tolerance by serial passage.

4.8.1.2 Hexaconazole Tolerant Culture

On the first DAT, highest mortality observed was 60 per cent, in non-selected *L. saksenae* which was significantly higher than that observed with selected culture where the mortality was 3.33 per cent. No mortality was observed in other treatments.

On the third DAT 100 per cent mortality was observed in non-selected culture which significantly varied from all other treatments. Mortality of 36.67 per cent was observed in selected culture and in selected + hexaconazole 5% EC 0.15% treatment, only 20 per cent mortality was noticed.

Fifth DAT, bugs treated with the selected exhibited 56.67 per cent mortality while it was significantly lower (36.67 per cent) in selected + hexaconazole 5% EC 0.15%. No mortality was observed in non-selected + hexaconazole 5% EC 0.15%, hexaconazole 5% EC 0.15% and control in any of the day.

Selected cultures when sprayed alone exhibited lower effectiveness (66.67 per cent mortality) than non-selected culture on the fifth DAT where 100 per cent mortality was observed. Selected cultures treated along with hexaconazole 5% EC 0.15% exhibited higher mortality compared to non-selected culture +

hexaconazole 5% EC 0.15%, where no mortality was observed which proves the tolerance induced by serial passage is feasible.

4.8.1.3 Pathogenicity of L. saksenae UV Tolerant Mutant

Pathogenicity assay was conducted to compare the pathogenicity of wildtype and LsUVM (*L. saksenae* UV mutant) against rice bug and observation were taken at 24 hours interval (Table 41).

On the first DAT, 54 per cent mortality was observed which was statistically on par with that of its wild type (64 per cent mortality). No mortality was recorded in control. Third DAT, highest mortality was observed in wild type *L. saksenae* which was on par with LsUVM mutant where per cent mortality of 98 per cent was observed

There was no improvement in pathogenicity of mutant, over the wild type.

4.8.1.4 Pathogenicity of L. lecanii UV Tolerant Mutant

The pathogenicity of *L. lecanii* mutants @ 10^7 spores mL^{-1} tested on brinjal mealybug is presented in Table 42.

Second DAT, no significant difference was observed across the treatments, with 3.75 to 10 per cent in LlUV1, LlUV2 and LL wild type (LlWT). Fourth DAT, nymphs treated with LlUV1 exhibited 76.25 per cent mortality. The corresponding death rate was 53.75 and 50 per cent in LlWT and LlUV2, respectively.

On the sixth DAT, mortality in LlUV1 remained same (96.25 per cent) while it increased to 80 per cent in LlWT which was on par with LlUV2 (73.75 per cent mortality). On the eighth DAT, mortality increased to 83.75 per cent in wild type which was similar to that noted in LlUV2 (73.75 per cent mortality) LlUV1 which was isolated at 30 min UVC exposure exhibited increase in virulence than other two test cultures. On the sixth DAT the LlUV1 caused highest mortality (96.25 per cent) in mealybug where others caused only 60 per cent mortality.

Table 41. Pathogenicity of UV tolerant strain of *Lecanicillium saksenae* against *Leptocorisa oratorius*

Treatments	Mortality at 24 h. interval (%)				
	Day1	Day2	Day3	Day4	Day5
WT Ls	64.00 (53.23)[a]	80.00 (64.154)[a]	100 (89.17)[a]	100 (89.17)[a]	100 (89.17)[a]
LsUVM	54 (47.35)[a]	88.00 (69.94)[a]	98.00 (86.08)[a]	98.00 (86.08)[a]	98.00 (86.08)[a]
Control	0 (0.286)[b]	0 (0.286)[b]	0 (0.286)[b]	0 (0.286)[b]	0 (0.286)[b]
CD (0.05)	(9.477)	(7.367)	(6.458)	(6.458)	(6.458)

LsUVM - *L.saksenae* UV mutant; WTLs - Wild type *L. saksenae* Values in parenthesis are arc sin transformed. Values sharing same alphabets in superscript are statistically on par based on ANOVA

Table 42. Pathogenicity of UV tolerant strain of *Lecanicillium lecanii* against *Coccidohystrix insolita*

Treatment (10^7 spore mL^{-1})	Mortality at 48 h interval			
	Day 2	Day 4	Day 6	Day 8
LlUV1	10.00(15.93)	76.25 (61.00)[a]	96.25 (82.02)[a]	100 (89.71)[a]
LlUV2	3.75 (9.76)	50.00 (45)[a]	73.75 (59.29)[b]	73.75 (59.29)[b]
WT Ll	5.00 (11.14)	53.75 (47.18)[a]	80.00 (63.75)[b]	83.75 (66.93)[b]
Control	0 (0.29)	0 (0.29)[c]	0 (0.29)[c]	0 (0.29)
CD (0.05)	NS	(8.91)	(8.79)	(6.48)

LlUV1- *L. lecanii* UV mutant one; LlUV2- *L. lecanii* UV mutant two; WTLl - Wild type *L. lecanii* NS - Non significant; Values in parenthesis are arc sin transformed.

Discussion

5. DISCUSSION

Microbial biopesticides occupy only 12 per cent of the India's total pesticide market, (DPPQ & S, 2021), owing to their less field persistence unlike the chemical pesticides. One among the major factors responsible for their insufficient field sustainability is the exposure to abiotic stresses. Upon field application, these microbes are exposed to an array of stress factors such as exposure to pesticides (Shapiro-Ilan *et al.,* 2002), UV radiation (Rangel *et al.,* 2004) and high temperature (Rangel *et al.,* 2006) each having some negative effect on their virulence and viability. Extremities in any of the above abiotic factors results in the failure of microbial pest control. Screening of isolates is essential to recognize competent entomopathogens as the biocontrol features are probably isolate-dependent (Kohl *et al.,* 2011). Validating a native isolate that have evolved to a geographical habitat can provide immense potential for biological management of relevant pests. Hence, the present investigation was focused to study the comparative tolerance of the native entomopathogenic fungus *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and the popularly used species *Lecanicillium lecanii* (Zimm.) Zare and Gams, to pesticides, temperature and UV radiation with an objective to develop tolerant strains.

5.1 PESTICIDE TOLERANCE

Integration of any fungal bioagent with an insecticide or fungicide requires a thorough knowledge of compatibility or tolerance between the two agents particularly with reference to growth, sporulation and germination.

Tolerance of *L. saksenae* and *L. lecanii* to old and new generation insecticides assessed *in vitro* in terms of growth, sporulation and germination is discussed below. To *L. saksenae*, the new generation insecticides flubendiamide 39.35% SC, chlorantraniliprole 18.5% SC, imidacloprid 17.8% SL and

thiamethoxam 25% WG were least inhibitive to their growth causing only 6.33 to 13.54 per cent inhibition at their recommended doses (Fig. 4).

In *L. lecanii*, imidacloprid 17.8% SL was noninhibitive with negligible (three per cent) reduction in growth, while flubendiamide 39.35% SC caused up to 17.23 per cent reduction. The effect of these new generation insecticides on the sporulation of fungi when studied it was seen that, there was only 12 to 16 per cent reduction in *L. saksenae* while in *L. lecanii* there was significantly higher inhibition (39.46 per cent). Germination inhibition was below 15 per cent with these new generation insecticides, in both the fungi (Fig. 5). There was only 4.33 per cent inhibition with thiamethoxam 25% WG in *L. saksenae* while it was 11 per cent in *L. lecanii.* These findings that prove the compatible or tolerant nature of entomopathogenic fungi to new generation insecticides are supported by various studies.

Non inhibitive effect of chlorantraniliprole 18.5% SC in this experiment is in corroboration with the findings of Sitta *et al.* (2009) in *Metarhizium anisopliae* (Metschn.) Sorokin and Vijayasree (2013) in *L. lecanii.*

Imidacloprid and thiamethoxam were reported to be exhibit less growth inhibition in *L. lecanii* and *Beauveria bassiana* (Bals.) Vuill. as substantiated in the experiments carried out by Filho *et al.* (2001), Rachappa *et al.* (2007) and Kakati *et al.* (2018). Thiamethoxam was reported to cause only 12.37 per cent growth inhibition in *B. bassiana* according to Neves *et al.* (2001). Both thiamethoxam and imidacloprid had no adverse impact on germination of *L. lecanii* as reported by Gurulingappa *et al.* (2011). Ummer and Kurien (2021) reported that imidacloprid 17.8% SL caused only 4.19 per cent inhibition in *L. lecanii.* On the contrary, perusal of literature reveals significant level of inhibitory action of thiamethoxam 25% WG in entomopathogenic fungi. Oliveira *et al.* (2003) reported 21.39 per cent sporulation inhibition in *B. bassiana*, while Akbar *et al.* (2012) observed 49.48 per cent inhibition in sporulation of *M. anisopliae*. Likewise, Vijayasree (2013) reported 65 per cent reduction in mycelial growth of *L. lecanii*.

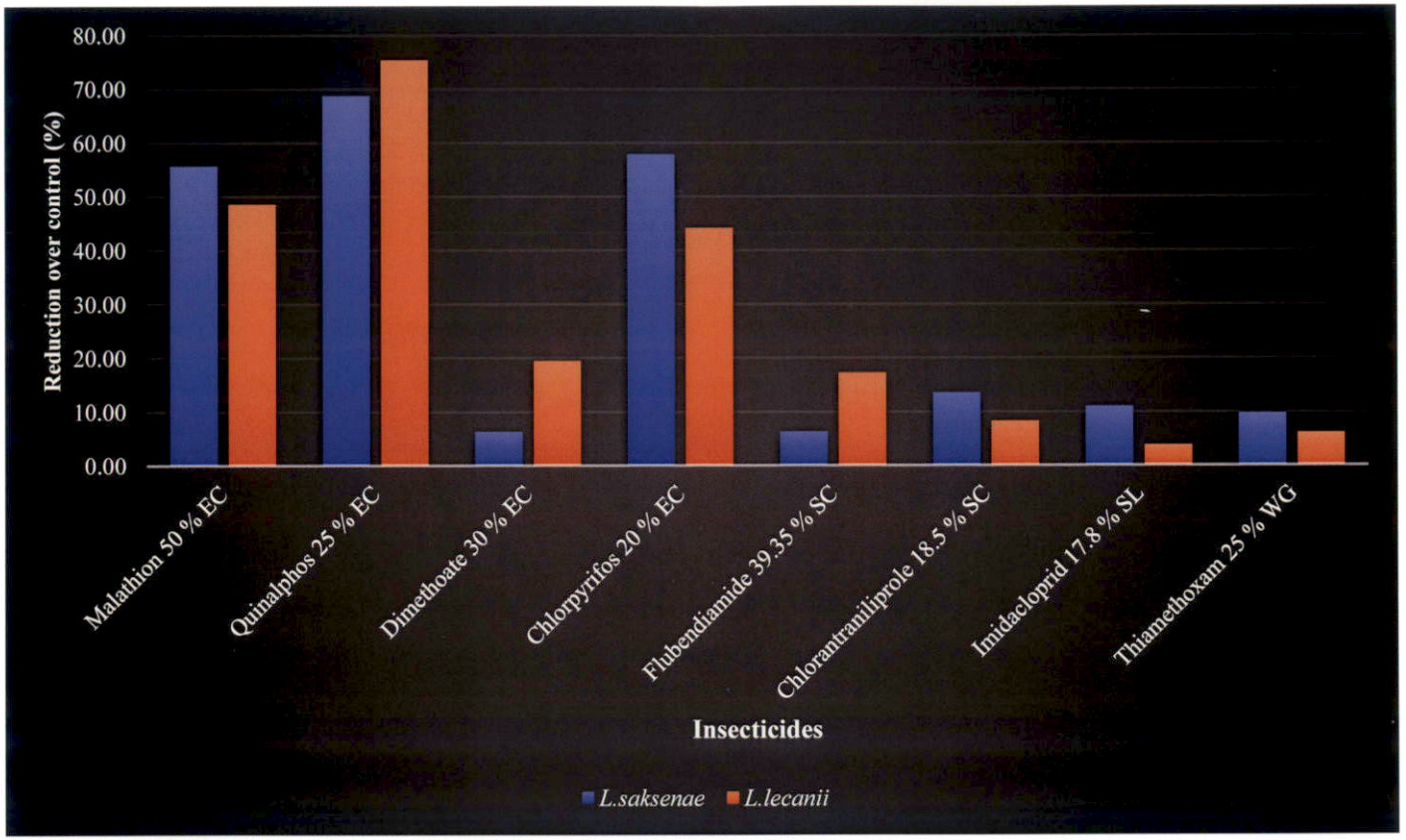


Fig. 4. Reduction in growth of *Lecanicillium saksenae* and *Lecanicillium lecanii* in recommended dose of insecticides

So also, Goncalves *et al.* (2019) reported that flubendiamide totally inhibited the growth, sporulation and germination of *M. anisopliae.*

Among the old generation insecticides tested, it was dimethoate 30% EC that was comparatively less inhibitory on growth parameters of both the fungi. Inhibition on growth was 6.33 per cent in *L. saksenae* and 19.46 per cent in *L. lecanii*. It caused 32.30 per cent inhibition in sporulation of *L. saksenae* while in *L. lecanii* the inhibition was more (51.33 per cent). Its negative effect on germination was very less in both the species (11 to 20 per cent). In the studies carried out by Armarkar and Chikte (2008) and Kakati *et al.* (2018), dimethoate 30% EC was found to be compatible to *L. lecanii* causing only 19.63 and 21.25 per cent inhibition in growth. However, it was reported to have adverse effect on the growth of *B. bassiana* with 59.25 per cent inhibition (Dhanya *et al.,* 2019).

The level of growth inhibition caused by other old generation insecticides such as malathion 50% EC, and chlorpyrifos 20% EC was to the tune of 44 to 57 per cent. These insecticides significantly deterred the fungi, causing 85 to 94 per cent inhibition in sporulation and 23 to 38 per cent inhibition to the conidial germination. Inhibitory nature of malathion 50% EC and chlorpyrifos 20% EC noted in this experiment is in corroboration with the earlier findings of Rani (2018) in *L. saksenae,* wherein she observed a significant reduction in the growth and sporulation. Additionally, the inhibitory nature of chlorpyrifos 20% EC and malathion 20% EC to other entomopathogens such as *B. bassiana* was confirmed in the studies conducted by Rachappa *et al.* (2007) where there was 58 per cent reduction in growth by malathion 50% EC and 69 per cent reduction by chlorpyrifos 20% EC. So also, Rajanikanth *et al.* (2010) observed a total inhibition in sporulation of *B. bassiana* with chlorpyrifos 20% EC. Further, Faraji *et al.* (2016) also reported total inhibition of growth and sporulation of *B. bassiana* and *M. anisopliae*, by chlorpyrifos 20% EC.

Quinalphos 25% EC was highly inhibitive to the growth (68 to 75 per cent), sporulation (99 per cent) and germination (47 to 54 per cent). Quinalphos

25% EC exhibited highest inhibition where there was 68 per cent reduction in *L. saksenae* and 75 per cent reduction in *L. lecanii.*

The inhibitory effect of quinalphos noted in this study is supported by the findings of various researchers. Further, Dhanya *et al.* (2019) reported more than 60 per cent growth inhibition in *L. lecanii.*

Rajinikanth *et al.* (2010) and Faraji *et al.* (2016) reported the non-compatibility of quinalphos 25% EC where there was a total inhibition in conidial germination of *B. bassiana.* Likewise, Babu *et al.* (2014) observed that quinalphos 25% EC affected the viability of *B. bassiana* by 51.10 per cent.

The reason for inhibition noted with many of the old generation insecticides as opined in the study carried out by Rani, (2000) in the entomopathogenic fungus *Fusarium pallidoroseum* (Cooke) Sacc, might be due the alteration in C: N ratio in the poisoned medium to non-ideal proportions. This may be the reason why fungi respond differently in different media into which other chemicals are added. Only if the carbon and nitrogen source is in the available form that can be metabolised by the fungi, it can grow and sporulate well. She further stated that quinalphos which partially inhibited sporulation, did not affect the growth, indicating that factors affecting growth may be different from those affecting sporulation.

As all the three parameters, the growth, sporulation and germination play a crucial role in inciting infection and self- perpetuation of an entomopathogenic fungus. Any one parameter alone cannot be used to determine the compatibility or tolerance. Therefore, compatibility status was determined by using Biological Index (BI), which take in account of all the growth parameters, enabling to classify them as 'compatible', with BI > 66; 'moderately toxic', with BI = 42 to 66 and 'toxic', with BI < 41. Accordingly, all the new generation insecticides tested were found to be compatible with both the fungi. The BI value ranged between 86 to 89 in *L. saksenae* and between 75 to 80 in *L. lecanii.*

Malathion 50% EC, quinalphos 25% EC and chlorpyrifos 20% EC were classified as 'toxic' to both the fungi, with BI ranging from 17 to 38. Only dimethoate 30% EC was found to be 'compatible' with BI 89 in *L. saksenae* and 69 in *L. lecanii*. Incompatibility of *L. lecanii* with chlorpyrifos 20% EC is in agreement with studies conducted by Abidin *et al.,* (2017) where it was found to be toxic to *B. bassiana* and *M. anisopliae* based on BI index (39.32 and 24.40 respectively).

In general, this study concludes that *L. saksenae* is highly tolerant to insecticides, being an indigenous isolate, compared to other entomopathogens such as *L. lecanii*, *B. bassiana* and *M. anisopliae*. This ability of *L. saksenae* to degrade pesticides was earlier reported by Pinto *et al.* (2012) who stated the *L. saksenae* PP0011 a native isolate of Portugal, degraded pesticides to an extent of 99.5 per cent.

Among the fungicides studied for tolerance, azoxystrobin 23% SC was comparatively less inhibitive to *L. saksenae* causing only 1.85 per cent growth inhibition (Fig. 6). Its impact on sporulation was adverse causing 56.61 per cent reduction. In *L. lecanii* the inhibition was significantly higher (41.76 per cent and 85.68 per cent) respectively, in growth and sporulation. However, the reduction in germination was only five per cent, in both the fungi. Silva *et al.* (2013) reported least inhibition in growth and sporulation of *M. anisopliae*. On the contrary, Zumaeta (2014) pointed out that azoxystrobin 300 ppm reduced the germination by 81 per cent and growth by 51 per cent.

Copper oxychloride 50% WP caused 100 per cent inhibition in the growth and sporulation of *L. saksenae*, whereas the corresponding values in *L. lecanii* were 35.00 and 91.17 per cent. In terms of germination its inhibition was at negligible level (< 2 per cent) in both the fungi. The inhibitory effect of copper oxychloride 50% WP on growth and sporulation of entomopathogenic fungi noted in this study is in accordance with the report of Olan and Cortez, (2003) who found that there was 79.24 per inhibition in *L. lecanii.* In *B. bassiana* it caused 45-55 per cent inhibition in sporulation (Rachappa *et al.,* 2007).

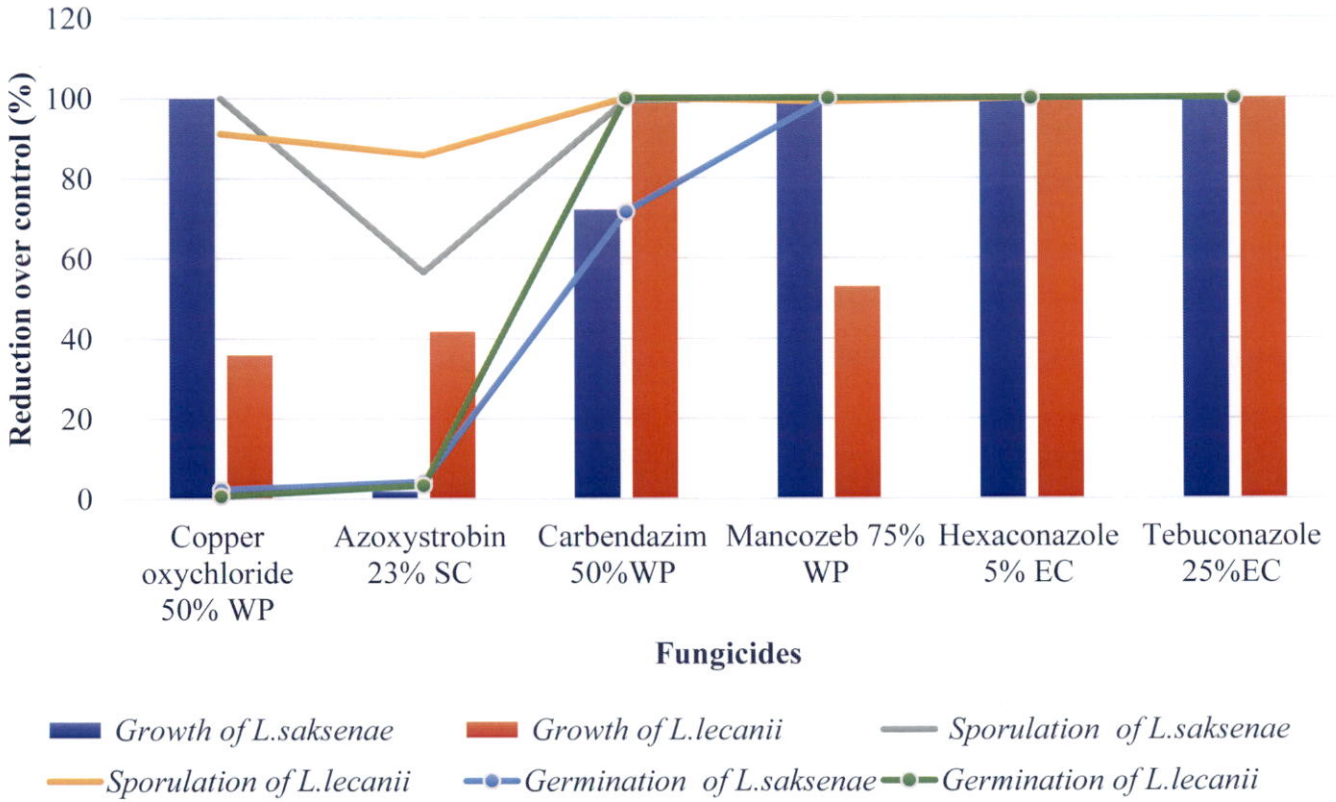


Fig. 6. Reduction in growth, sporulation and germination of *Lecanicillium saksenae* and *Lecanicillium lecanii* in recommended dose of fungicides

In addition, according to Shoeb *et al.* (2021) it inhibited the germination of *B. bassiana* and *M. anisopliae* at 0.2 to 1% which was two to ten times higher than the recommended dose.

In this investigation, it was interestingly noted that in some culture plates there was a unique pattern of growth of *L. saksenae* in the medium poisoned with copper oxychloride 50% WP 0.1 %, 30 DAI. This when subcultured on non-poisoned PDA the growth reverted back to normal pattern. The phenomenon may be due to better adaptability of the fungus to copper oxychloride and to surpass the adverse conditions.

Mancozeb 75% WP was found to be highly poisonous to both the fungi. It caused 52.94 per cent growth inhibition, 99.04 per cent sporulation inhibition and hundred per cent germination inhibition of *L. lecanii.* Whereas, in *L. saksenae*, all the growth parameters were completely inhibited. Mancozeb is known to affect cellular respiration, interrupting the Krebs cycle in multiple stages, thus it inhibitory to the growth and as well as germination of fungi (Liñan, 1997). Gonzalez *et al.* (2012) found that mancozeb 75% WP @ 2000 mg kg^{-1} exhibited a spore load of 2.3 x 10^7 mg kg^{-1} in *L. lecanii*, while there was 34.30 per cent growth inhibition. It was also reported to cause complete inhibition in the germination of *Lecanicillium muscarium* (Petch) Zare & W. Gams by Ali *et al.* (2013), in *Isaria fumosorosea* Wize, by Bernal *et al.* (2014) and *B. bassiana* by Faraji *et al.* (2016) in *B. bassiana* and *M. anisopliae.*

Carbendazim 50% WP caused 72.19, 99.44 and 71.67 per cent inhibition to the growth, sporulation and germination of *L. saksenae* respectively, while the rate of inhibition was 100 per cent in *L. lecanii.* This complete inhibition of *L. lecanii* is by carbendazim is in concordance with the observation of Krishnamoorthy and Visalakshi (2007) and Ummer and Kurien, (2021). It is notable that *L. saksenae* was able to grow and sporulate in carbendazim poisoned medium and there are no earlier report of any entomopathogenic fungi growing on carbendazim poisoned medium. This tolerant nature of *L. saksenae* to the commonly used fungicide is a boon in IPDM of pests and diseases.

Hexaconazole 5% EC and tebuconazole 25% EC exhibited 100 per cent inhibition of growth, sporulation and germination of both *L. saksenae* and *L. lecanii*. Similar inhibitory properties of hexaconazole 5% EC were reported by Raj *et al.* (2011) in *B. bassiana*, Lavanya and Matti, (2020) and Johnson *et al.* (2020) in *M. anisopliae* where there was a total arrest. Completely inhibiting nature of tebuconazole 25% EC was reported in *L. lecanii*, *B. bassiana*, *M. anisopliae* and *I. fumosoroseus* by Loureiro *et al.* (2002) and in *L. lecanii* by Reddy *et al.* (2018).

Compatibility status when assessed for fungicides it was seen that only azoxystrobin 23% SC was compatible with *L. saksenae* with BI 74, while it was moderately toxic to *L. lecanii* with BI value, 46. Compatibility of azoxystrobin 23% SC was earlier reported by Silva *et al.* (2013) where he noted a BI of 72.6 in *M. anisopliae*. The BI values for copper oxychloride 50% WP was 10 and 44 respectively in *L. saksenae* and *L. lecanii,* indicating that it was highly toxic to *L. saksenae* and moderately toxic *to L. lecanii.* Other fungicides *viz.*, carbendazim 50% WP, mancozeb 75% WP, hexaconazole 5% EC and tebuconazole 25% EC was "toxic" to both the fungi. Bartlett *et al.* (2002) suggested that the toxicity of tebuconazole and hexaconazole the triazole fungicides, is due to ergosterol biosynthesis inhibition, consequently preventing the formation of fungal cell membrane. Mancozeb which found to be toxic in this study was earlier reported to be moderately toxic to *L. lecanii* by Gonzalez *et al.* (2012). This variation may be attributed to the fact that in their study, only growth and sporulation was taken into account for computing compatibility index based on the scale of classification put forth by International Organisation of Biological Control.

With a view to develop strains tolerant to incompatible insecticides and fungicides, the Highest Tolerant Dose (HTD) of each of these was determined based on growth, sporulation and germination. Among the insecticides, quinalphos 25% EC was selected to induce tolerance in both the fungi, which had

HTD of 0.1 %, by subculturing fungi for 10 successive generations on media poisoned with HTD of quinalphos 25% EC. As there was no improvement in any of growth parameters in the "selected" culture compared to the "non selected culture" the induction was found to be not successful, in both the fungi.

In *L. saksenae,* the HTD was assessed for all the fungicides except azoxystrobin 23% SC which was compatible. It was 1% in carbendazim 50% WP and 0.015% in hexaconazole 5% EC. In mancozeb 75% WP and tebuconazole 25% EC there was neither growth nor sporulation even at $1/10^{th}$ of recommended dose. Hence, they were eliminated from further studies.

In *L lecanii,* the HTD was assessed for all the fungicides as they were incompatible. In copper oxychloride 50% WP and azoxystrobin 23% SC, the HTD was 1.0% and 0.5% respectively, where it exhibited minimum growth, sporulation and germination. In mancozeb 75% WP, growth and sporulation terminated at 0.6 %. Therefore, 0.30 % was chosen to induce tolerance. Even though at 0.03 % ($1/10^{th}$ of recommended dose) no germination was observed, an attempt was made to induce tolerance based on HTD selected for growth and sporulation. Carbendazim 50% WP, hexaconazole 5% EC and tebuconazole 25% EC showed neither growth nor germination even at $1/10^{th}$ of recommended dose, hence no attempt was made to induce tolerance.

The induction of fungicide tolerance in *L. saksenae* resulted in two strains *i.e.,* one carbendazim tolerant strain and one hexaconazole tolerant strain. The relaxed and selected cultures of carbendazim tolerant strain exhibited 126.63 per cent and 76.92 per cent increase in colony growth (Fig. 7) and in terms of sporulation, the improvement was 381.03 per cent in the selected and 931.03 per cent in the relaxed culture. In germination, the percentage increase was 203.76 in selected and 206.41 in the relaxed culture. Hexaconazole 5% EC tolerant strain was able to tolerate the recommended dose (0.15 %), while the non-selected culture was able to tolerate only $1/10^{th}$ of the recommended dose (0.015 %). Therefore, it is concluded that the tolerance level could be increased by 10 times in *L. saksenae,* by induction.

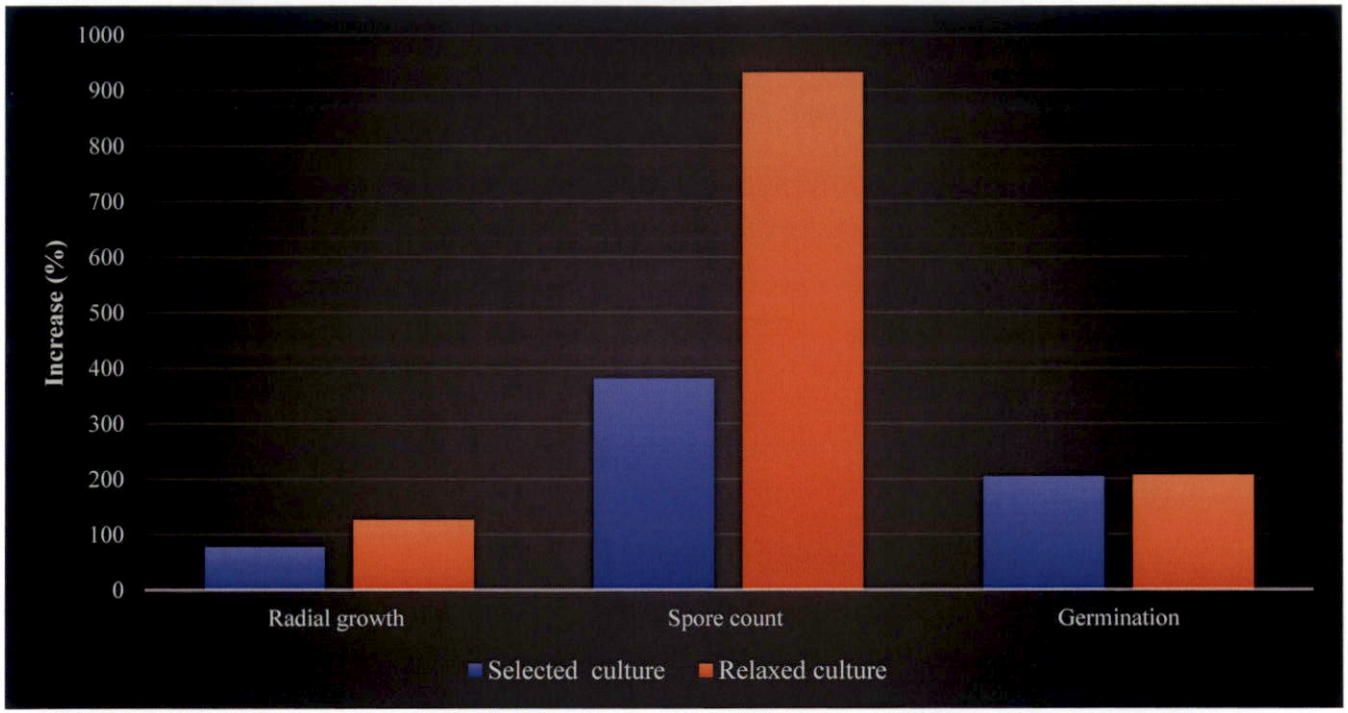


Fig. 7. Comparison of carbendazim tolerant strain of *Lecanicillium saksenae* with non-selected culture

These results indicate that artificial selection can improve fungicide tolerance in *L. saksenae*. This finding is in agreement with that of Shapiro-Ilan *et al.* (2002), where artificial selection was reported to be a success in *B. bassiana*. In their study, the GHA strain was able to develop tolerance to fungicides dodine, fenbuconazole and triphenyltin hydroxide after successive passage through HTD for ten generations.

Shapiro-Ilan *et al.* (2011) attempted to tolerance development in *B. bassiana* and *Metarhizium brunneum* Petch and the results revealed that artificial selection over 10 generations enhanced the tolerance to fenbuconazole and triphenyltin hydroxide, in both the fungi. Nilamudeen and Sudharma (2021) reported that *B. bassiana* (Bb5) and *M. anisopliae* (Ma4) tolerated four times the field dose of acephate 75% WP, eight times the dose of chlorantraniliprole 18.5% SC and thiamethoxam 25% WG, through artificial selection over 10 generations. Such enhancement of tolerance may be substantiated by the concept of phenotypic plasticity, where an individual genotype is able to produce different phenotypes when exposed to harsh environmental conditions, as opined by Pigliucci *et al.* (2006).

In *L. lecanii,* tolerance induction was attempted with copper oxychloride 50% WP, azoxystrobin 23% SC and mancozeb 75% WP. In copper oxychloride and azoxystrobin there was no improvement in tolerance when passed through 10 generations in HTD.

The comparison of the selected, relaxed and non-selected cultures revealed no significant difference in growth parameters. In mancozeb poisoned media, the growth terminated at sixth generation, with no indication of tolerance. Shapiro-Ilan *et al.* (2002) while working with mixed strain of *B. bassiana* could not achieve any enhancement to dodine, fenbuconazole and triphenyltin hydroxide tolerance, even after 10 serial passages in HTD.

5.2 TEMPERATURE TOLERANCE

Temperature is a crucial factor that determines the growth and sporulation of an entomopathogenic fungus during mass culturing and field application. Tolerance of *L. saksenae* and *L. lecanii* to different temperature regimes was studied based on their growth, sporulation and germination. The optimum temperature for growth and sporulation was found to be 26 °C in both the fungi. This finding agrees with those of Hong and Kim (2007) and Vu *et al.* (2007) who proved that 25 °C is the optimum temperature for *Lecanicillium* species.

Khan *et al.* (1993) reported sporulation to the tune of 10^{7} to 10^{8} spores mL^{-1} in *B. bassiana* and *M. anisopliae* at 26 °C. So also, Thomas and Jenkins (1997) and Arthurs and Thomas (2001) reported that 26 to 28 °C is the optimum temperature for growth and sporulation of *Metarhizium flavoviride* Gams and Rozsypal and *Metarhizium acridum* (Driver& Milner)

As the temperature was increased above 30 °C, substantial decrease in growth was observed in both the fungi (Fig. 8). However, the growth in *L. saksenae* was more when compared to that in *L. lecanii*. At 30, 32, 34 and 36 °C a relative growth of 74.79 per cent, 63.84 per cent, 28.52 per cent and 16 per cent was noted in *L. saksenae* which was significantly higher than that in *L. lecanii* where it was 66.32 per cent, 33.67 per cent, 23.65 per cent and 0 per cent, respectively. Such steady decrease in growth with increase of temperature has earlier been reported by Kope *et al.* (2008) in *Lecanicillium longisporum* (Petch) Zare and Gams, *L. muscarium and Lecanicillium pissodis* Kope & I. Leal. where they noted a drastic reduction in growth with increase in temperature above 30 °C.

Conidial production is one of the key traits in the selection of fungi for biological control (Hall, 1984; Drummond and Heale, 1988). In this study, temperature was not found to affect sporulation of *L. saksenae* up to 34 °C (10^{7} spores mL^{-1}), whereas in *L. lecanii* there was an exponential decrease at 32 °C and 34 °C where the count was 7.4×10^{6} and 3.0×10^{5} spores mL^{-1},

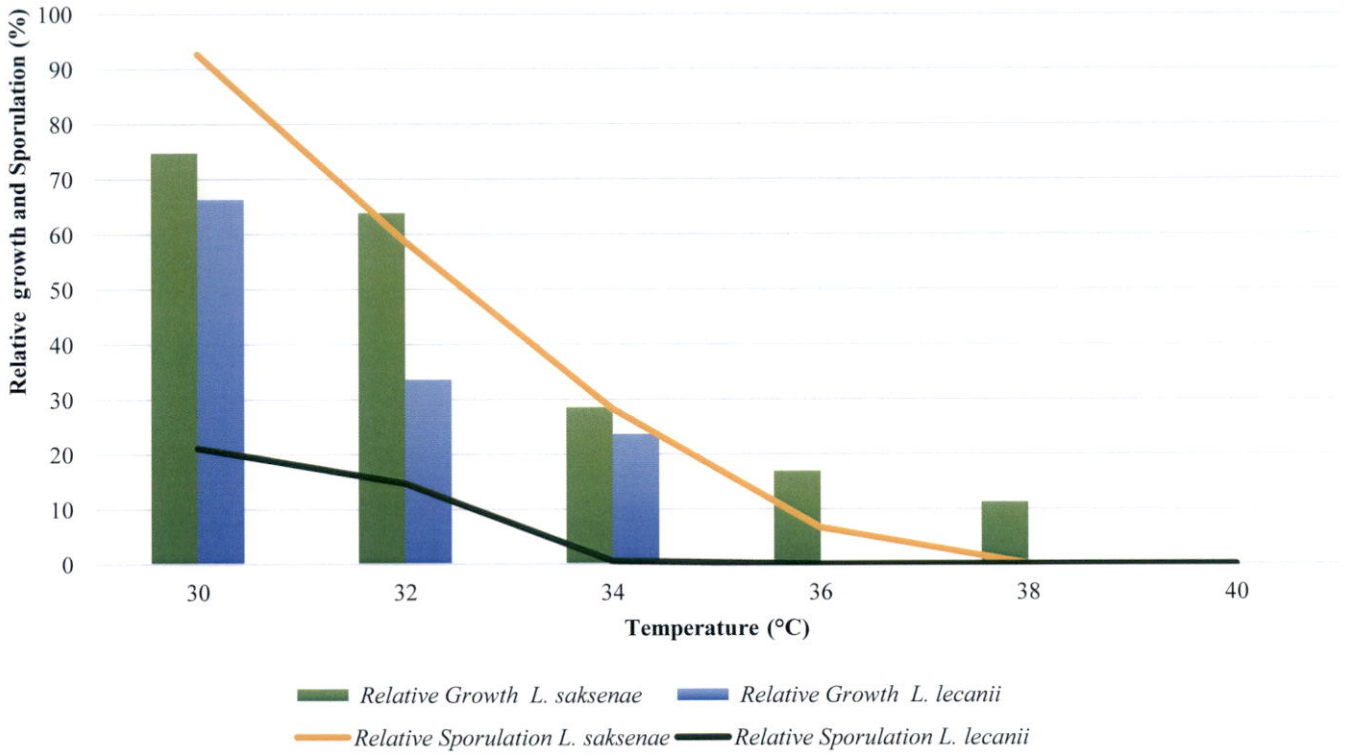


Fig. 8. Relative growth and sporulation of *Lecanicillium saksenae* and *Lecanicillium lecanii* at different temperature regimes

respectively. At 36 °C, *L. saksenae* yielded 3.1 x 10^6 spores mL^{-1} while in *L. lecanii* the sporulation was arrested.

In the studies conducted by Rivas *et al.* (2013), with *L. lecanii*, *L. longisporum* and *Lecanicillium attenuatum* Zare & W. Gams it was revealed *L. lecanii* is thermo sensitive as there was no conidiation at 32 °C, whereas the other two species exhibited 10^7 conidiation. Khan *et al.* (1993) observed that *M. anisopliae* was able to sporulate (10^7 spores mL^{-1}) at 35 °C. So also, Teja and Rehman, (2016) reported that *M. anisopliae* yielded 10^7 spores mL^{-1} at 32 °C.

In terms of germination, *L. saksenae* exhibited higher germination rate at all the test temperatures compared to *L. lecanii* (Fig. 9). At 26 °C, 100 per cent germination was observed in both the fungi, 48 h of treatment. This observation is in accordance with those of Burges and Hussey (1981) and Rivas *et al.* (2013) who reported 100 per cent germination of *L. lecanii* at 25 °C.

At higher temperatures (30, 32, 34 and 36 °C) *L. saksenae* exhibited 85 to 100 per cent germination at 48 h, whereas *L. lecanii* exhibited only 13 to 77 per cent germination. At 38 °C, *L. saksenae* the germination rate significantly reduced to 33.33 per cent while the corresponding value in *L. lecanii* was 2.33 per cent. Even at 40 °C *L. saksenae* could germinate (24.33 per cent). This difference in temperature tolerance between the species may be substantiated by findings of Zumin *et al.* (2018), where they attributed the variation in thermotolerance of two fungi to the variation in expression of heat shock protein (Hsp) in them.

The aforesaid results reveal that *L. saksenae* isolated from soils of Vellayani, Thiruvananthapuram, Kerala is tolerant to the tropical temperature regime.

Kope *et al.* (2008) and Rivas *et al.* (2013) while studying the responses of *Lecanicillium* spp. to environmental conditions opined that each isolate should be treated as a separate biological entity rather than generalising its features for the genus or species. They also asserted that *Lecanicillium* spp. exhibit great

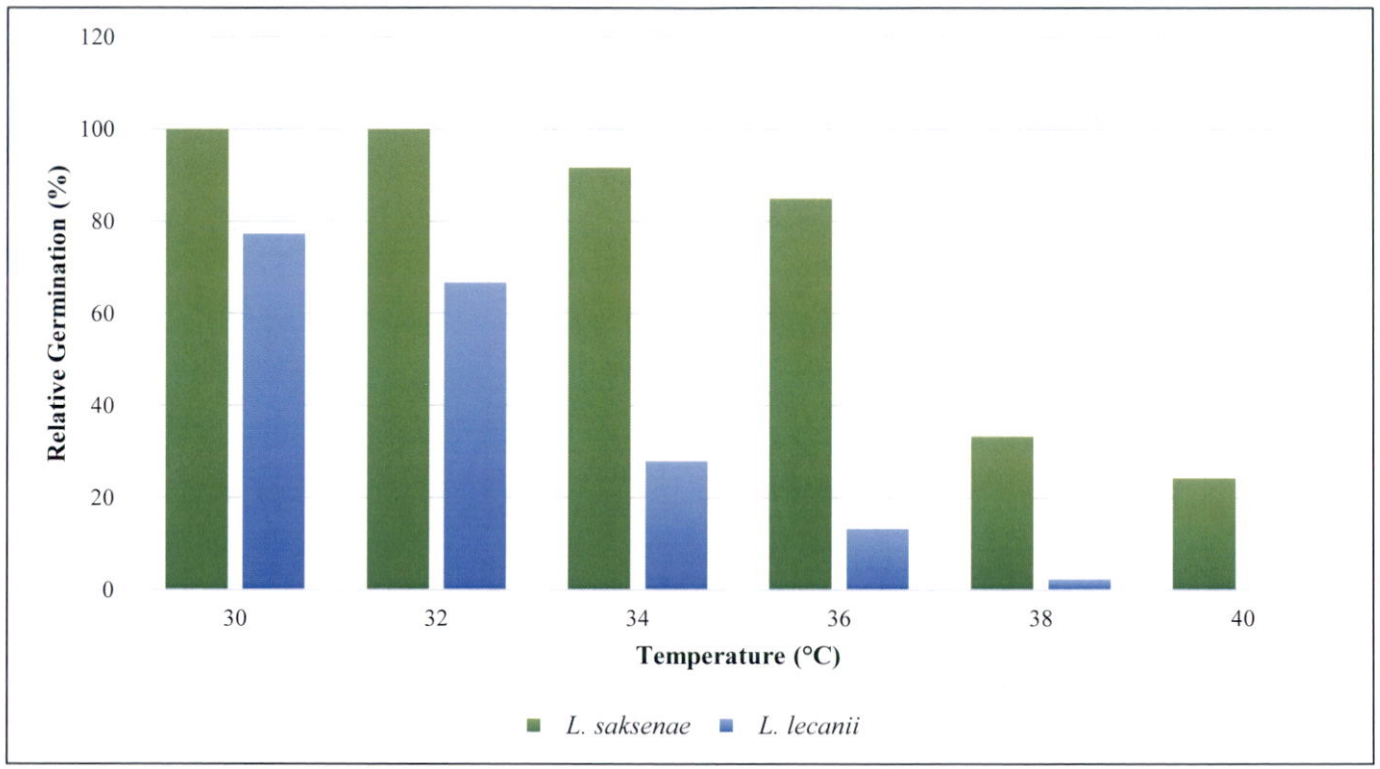


Fig. 9. Relative germination of *Lecanicillium saksenae* and *Lecanicillium lecanii* at different temperature regimes

global biodiversity and the geographical isolates naturally adapt to the regional temperature and humidity levels.

Attempt to induce temperature tolerance in order to develop tolerant strains was carried out as the next phase of the study by continuously sub culturing *L. saksenae* and *L. lecanii* at Highest Tolerant Temperature (HTT). The results revealed that *L. saksenae* was able to tolerate HTT of 36 °C up to sixth generation while *L. lecanii* was able to tolerate upto 34 °C for four generations after which the cultures failed to grow. Therefore, the attempt was unsuccessful. However, *L. saksenae* was able to tolerate HTT for three more generations than *L. lecanii.*

Another attempt was made to induce thermotolerance by natural selection where the germinated fungal spores were exposed to 50, 60, 70 and 80 °C for five, 10 and 20 min. in hot water bath. Nearly 30 colonies obtained at different exposures were again re-exposed to higher temperature. In this attempt a colony tolerating 80 °C for five min was obtained in *L. lecanii.* However, this colony was morphologically different than the wild type *L. lecanii* (original mother culture) and exhibited crumpled growth. Therefore, the second attempt also failed. Consequently, it is concluded that the induction of temperature tolerance by artificial selection is not a feasible method to obtain a thermotolerant strain

Majority of the studies related to induction of temperature tolerance, either used genetic engineering or mutation studies with physical mutagen (UV or gamma) or chemical mutagen (EMS mediated). On the other hand, fungi possess complex regulatory circuits that intimately control cellular growth and metabolism. Consequently, single or even multiple mutations often fail to produce the desired results. To overcome the undesirable effect of above mentioned methods a new method was formulated , based on the study of De Crecy *et al.* (2009). In that study, they used the principle of evolution by natural selection which involved highly advanced instruments involving Evolugator™ which is a newly developed automated continuous culture method. There was no access to such sophisticated automated equipment to carry out the present investigation. Therefore, only principle was adapted in this experiment.

5.3 TOLERANCE TO UV RADIATION

Entomopathogenic fungi are in general very sensitive to UV radiation. The UV tolerance of *B. bassiana* (Inglis *et al.*, 1995; Morley- Davies *et al.*, 1996; Huang and Feng 2009; Posadas *et al.*, 2012), *I. fumosorosea* (Fargues *et al.*, 1996), *M. acridum* (Davies *et al.*, 1996; Fargues *et al.*, 1996; Braga *et al.*, 2001b), and *M. anisopliae* (Fargues *et al.*, 1996; Braga *et al.*, 2001a) have been extensively researched. Other less investigated species included *Aphanocladium album* (Preuss) Gams, *L. lecanii, Simplicillium lanosoniveum* (van Beyma) Zare & Gams (Braga *et al.*, 2002).

UVA radiation with a wave length of 315 to 400 nm is the most prominent in the UV spectrum comprising 95 per cent of total UV spectrum reaching the earth surface (Braga *et al.*, 2001a). Because of the harmful effects of UVA in sunlight, this component of the spectrum was given due importance in this strain selection programme.

In this study, UVA was found to affect the growth, germination and sporulation of the test fungi significantly. Growth was significantly affected when exposed for more than one hour. When exposed for two hours, there was 50 per cent reduction in the colony size in *L. saksenae,* while in *L. lecanii* the corresponding time recorded was three hours. Furthermore, there was 50 per cent reduction in sporulation when *L.saksenae* was exposed for three hours, whereas in *L. lecanii* even one hour exposure reduced the sporulation to half of the control (Fig. 10). When exposed for four hours, the decrease in sporulation was same in both the fungi with a narrow difference of five per cent between them.

Exposure of spores to UVA severely inhibited germination as well (Fig. 11). Even with 10 to 30 min exposure, there was 74 per cent inhibition in *L. saksenae,* while it was lesser (68 per cent) in *L. lecanii*. Exposure for one hour and more resulted in more than 80 per cent inhibition in both the species.

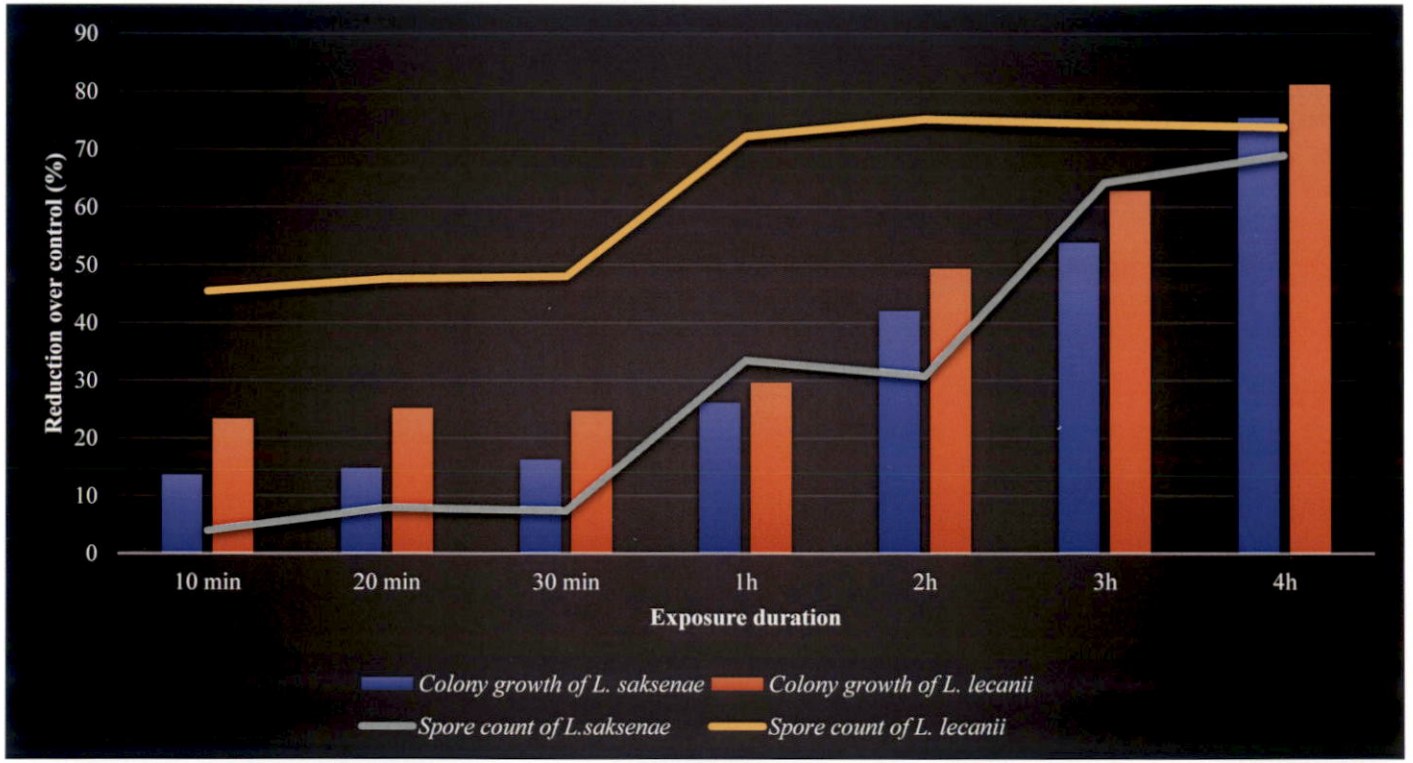


Fig. 10. Reduction of growth and sporulation of *Lecanicillium saksenae* and *Lecanicillium lecanii* under UVA irradiance

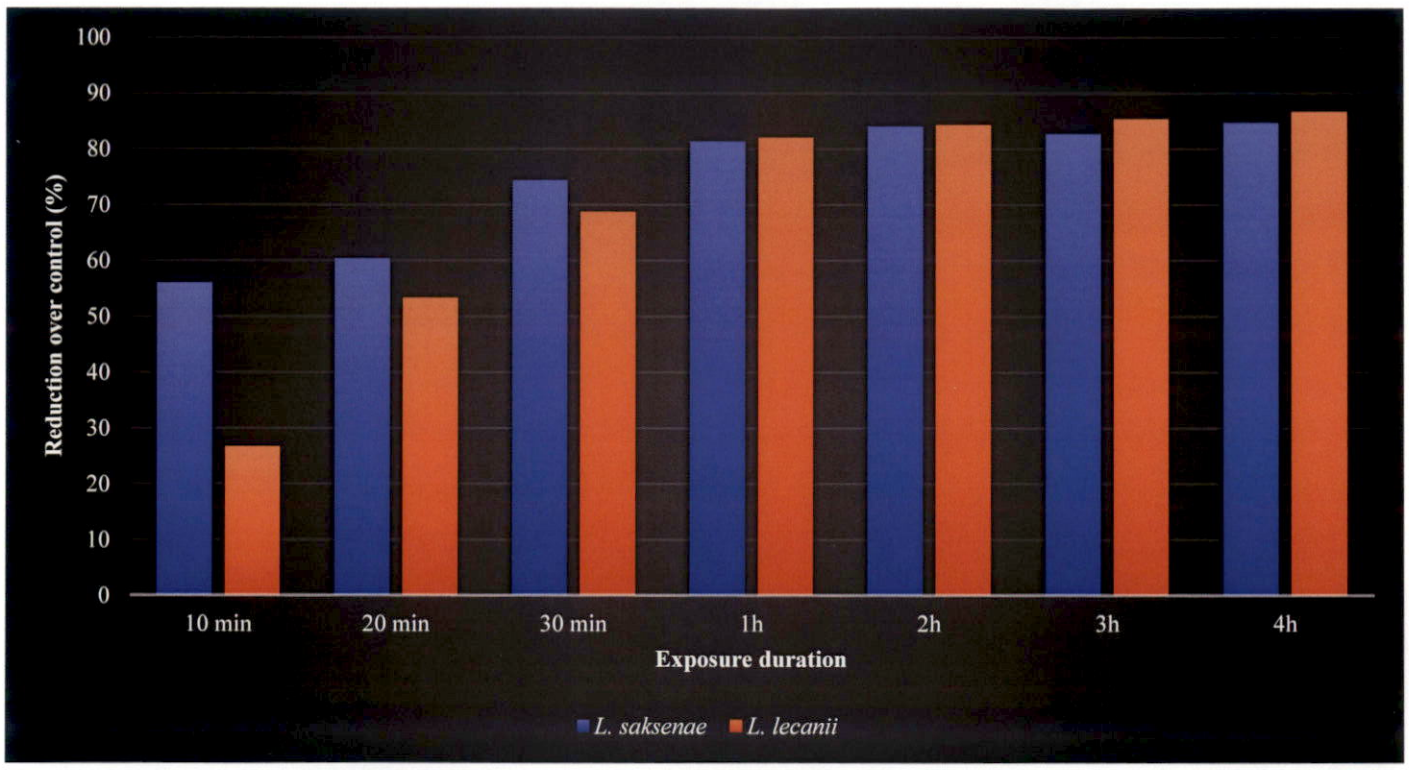


Fig. 11. Reduction in germination of *Lecanicillium saksenae* and *Lecanicillium lecanii* under UVA irradiance

Fargues *et al.* (1997) observed that three hour exposure to UVA resulted in 90 per cent germination inhibition in *Paecilomyces fumosoroseus* Wize. Likewise, Braga *et al.* (2001b) reported that exposure of *M. anisopliae* to UVA for three and four hours caused 20 and 40 per cent germination inhibition, whereas there was no inhibition when exposure duration was one or two hours, which is contrary to the findings of this study. This differential response of *M. anisopliae* may be due to the dark pigmented conidia which has rendered protection to UVA suggested by Ignoffo and Garcia (1992); Braga *et al.*(2005); Chelico *et al.* (2006); Nascimento *et al.* (2010).

Braga *et al.* (1999, 2001b) accounted the deleterious effect of UVA on germination of fungi, to the production of reactive oxygen species, ROS (unstable molecule that contains oxygen and that easily reacts with other molecules), which in turn damages the DNA and inhibit the conidial survivability. Further, they explained that the fungus that sustains in the environment and exposed to UV will be producing germlings (conidia) which is again exposed to the external harsh conditions during its germination process on the insect cuticle.

UVB type is very harmful to fungal propagules, significantly affecting their survival and efficacy against insects in the environment (Inglis *et al.,* 2001; Fernandes *et al.,* 2015; Acheampong *et al.,* 2020, Coucerio *et al.,* 2021).

In present study, when exposed for two hours, there was 50 per cent reduction in the colony size in both the fungi, while it was 70 per cent when the exposure time was increased to four hour (Fig. 12). In a study conducted by Braga *et al.* (2001a) it was revealed that even one hour exposure of *M. anisopliae* to UVB resulted in the same level of reduction in colony growth (50 per cent and 70 per cent).

When the exposure durations were short, ranging from 10 to 60 min, *L. saksenae* exhibited more growth (25 to 30 per cent) than *L. lecanii*. With two hour exposure, it had 10 per cent more growth than *L. lecanii.*

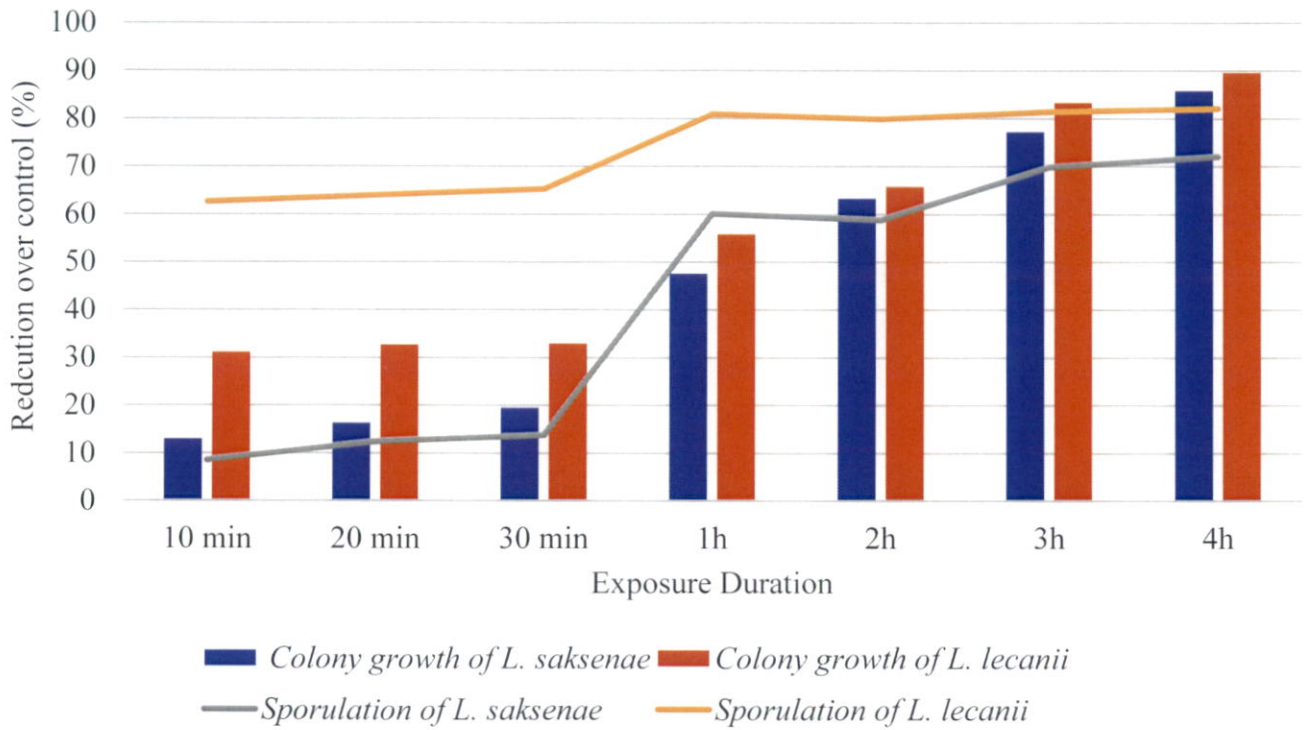


Fig. 12. Reduction in growth and sporulation of *Lecanicillium saksenae* and *Lecanicillium lecanii* under UVB irradiance

The above results indicate that, even though the growth reduction over control was similar in both the species, *L. saksenae* is capable to grow faster which may render an advantage in inciting infection.

UVB radiation did not cause any exponential decrease in sporulation. When exposed for 10, 20 and 30 min, there was only 10 per cent reduction in *L. saksenae* whereas it was above 50 per cent in *L. lecanii*. Longer exposure for more than one hour, resulted in same extent of reduction in both the species (70 to 80 per cent).

Germination was found to be severely inhibited by UVB. Short exposures of 10 min and 20 min reduced germination by 50 per cent in *L. saksenae* as well as *L. lecanii*. This observation is in concurrence with the report of Fargues *et al.* (1997), where 15 min of UVB exposure decreased the conidial viability by 50 per cent in *P. fumosoroseus*. In the same study, they reported 100 per cent inhibition at 30 min exposure which is contradicting with the finding of the present study, in which there was 25 to 30 per cent germination after 30 min of exposure, indicating a better level of tolerance of the genus *Lecanicillium* to *Paecelomyces*. Rodrigues *et al.* (2016) reported that 10 min of UVB exposure reduced the germination of *M. anisopliae* and *B. bassiana* by 90 per cent and no germination was observed at 20 min of exposure.

Many researchers have observed a decreasing trend in germination with the increase in exposure duration from one hour to four hours (Braga *et al.*, 2002; Fernandes *et al.*, 2007, Coucerio *et al.*, 2021). Some of the reports even suggest that four hour exposure can completely inhibit the germination of *B. bassiana* and *M. anisoplaie* (Braga *et al.*, 2001a, 2002; Bernardo *et al.*, 2020). But in the present study, with one hour exposure there was 20 per cent germination in both the fungi and the rate of decrease was less *i.e.*, even after four hour exposure there was 15 per cent germination as indicated in Fig. 13. This variation in result compared to earlier researchers may be due to the photoreactivation capability of the genus *Lecanicillium* rendering tolerance to UV.

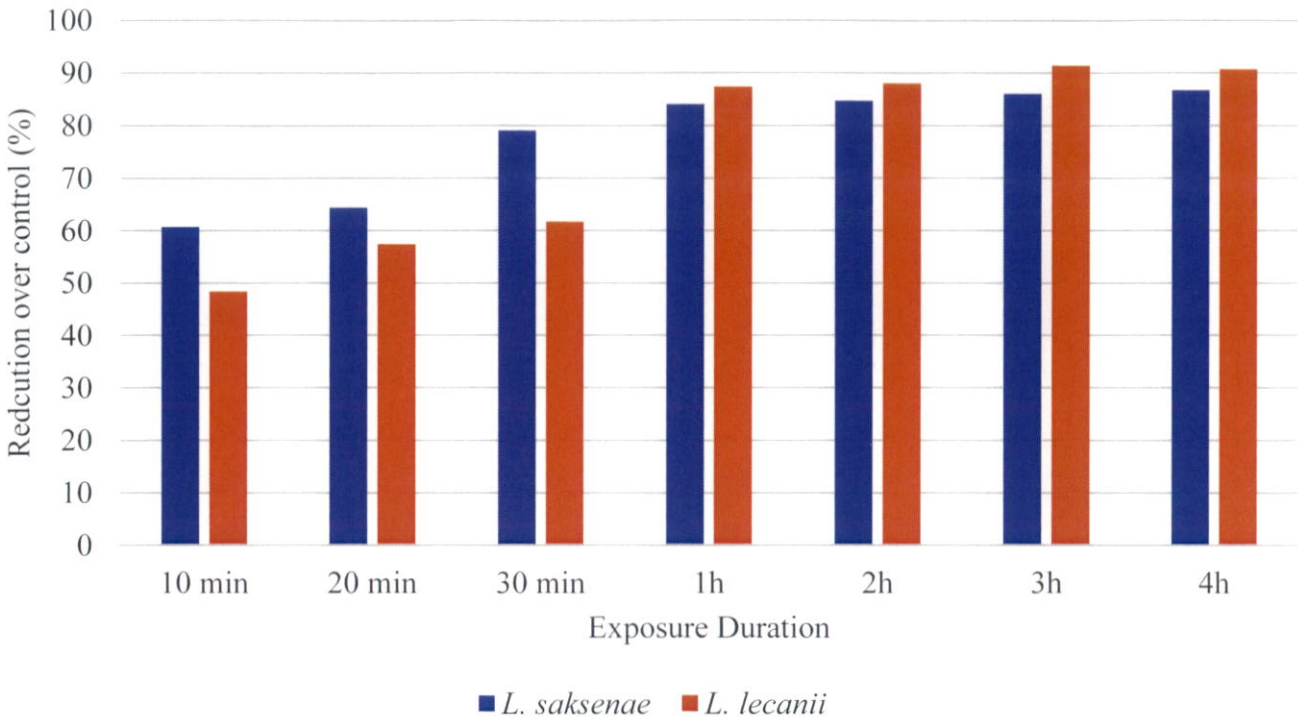


Fig. 13. Reduction in germination of *Lecanicillium saksenae* and *Lecanicillium lecanii* under UVB irradiance

Another reason cited by Braga *et al.* (2001a) is that, the conidia might undergo cell repair mechanisms during the initial four hours of exposure during which germ tube initiation and germination is initiated. It's very likely that, the decreased tolerance observed from sixth hour is associated with the beginning of DNA duplication. (Schmit and Brody,1976, Van Etten *et al.,* 1983).

In this experiment, delayed conidial germination was observed at 10, 20 and 30 min of exposure, where an increase of eight to 10 per cent was observed at 48 h, compared to 24 h. These results are supported by findings of Braga *et al.* (2001a) and Fernandes *et al.* (2007) where, germination of *B. bassiana* and *M. anisopliae* was reported to increase after 48 h of treatment.

Braga *et al.* (2002) opined that this delay in germination may be attributed to the repair systems such as segregation of fractured chromosomes and duplication of damaged genetic material which occurs in the time delay.

Therefore, the study on UV tolerance concludes that, *L. saksenae* exhibits higher colony growth, sporulation and germination than *L. lecanii* at various exposure duration of UVA and UVB.

The above results on UV tolerance ascertained the UV sensitivity of both the fungi. To obtain a UV tolerant strain UV mutagenesis study was carried out using UVC radiation (< 254 nm) using method described by Zhao *et al.* (2016). As a result, one UV mutant in each fungi were obtained.

L. saksenae mutant obtained by 20 min UVC exposure (LsUVM) exhibited increased tolerance to UVA and UVB radiation compared to the wild *L. saksenae.* LsUVM exhibited 25 to 50 per cent higher growth and 38 to 50 per cent higher germination in UVA irradiance while in UVB irradiance the corresponding values were 13 to 47 per cent and 13 to 41 per cent respectively in wild *L. saksenae.*

UV mutant of *L. lecanii* obtained at 30 min exposure to UV C (LlUVM1) also exhibited increased tolerance with 29 to 75 per cent increase in growth and 41 to 58 per cent increase in germination, under UVA irradiance. Under UVB irradiance also LlUV1 exhibited 26.54 to 54 per cent and 40.5 to 50 per cent higher growth and germination respectively when compared with wild *L. lecanii.*

Zhao *et al.* (2016) and Huang *et al.* (2021) evolved UV mutants of *M. anisopliae* and *Metarhizium lepidiotae* (Driver & Milner) after 40 min exposure, which in turn exhibited higher tolerance to UV radiation and increased virulence on diamond back moth, *Plutella xylostella* L.

The increase in UV tolerance exhibited in *B. bassiana* was attributed to a variety of transcription factors (TFs) such as CreA and Msn2 and modulators which are linked with C : N utilization in response to stress and virulence (Luo *et al.,* 2014, Ying *et al.,*2014).

5.4 MOLECULAR CHARACTERS OF TOELERANT STRAINS

Repetitive element sequence-based PCR (REP-PCR) is a new method that differentiates microbes by using primers complementary to interspersed repetitive consensus sequences, enabling amplification of diverse-sized DNA fragments consisting of sequences between the repetitive elements (Versalovic *et al.,* 1991 and 1994; Olive and Bean ., 1999). Trombert *et al.* (2007) revealed that REP- PCR could be used to study UV induced changes on DNA in bacteria and the use of a single pair of primers with a unique PCR reaction are enough to differentiate strains present in UV treated samples.

REP-PCR analysis of fungicide tolerant strains of *L. saksenae* developed in this study revealed the genotypic difference between mother culture, selected and non-selected culture. The similarity coefficient reveals that hexaconazole tolerant strain and carbendazim tolerant strain were 41.67 per cent and 37.5 per cent dissimilar to mother culture. This polymorphism is supported by the finding of Joseph (2014) who revealed that RAPD analysis of carbendazim tolerant strain of *B. bassiana* and *M. anisopliae* exhibited 83 per cent and 38 per cent

polymorphism, compared to mother culture. Whereas, in the report of Nilamudeen (2015) a contradictory result was seen, where no molecular variation was observed in RAPD analysis of insecticide tolerant culture of *B. bassiana* and *M. anisopliae* except minor polymorphism of 1.61 per cent exhibited in *B. bassiana.*

Genetic stability of strains developed in this study could be confirmed as the relaxed and selected cultures did not show any molecular variation. Further it could be proved that removal of selection pressure (fungicide) has not caused any loss of genetic improvements.

The REP- PCR analysis of UV mutants revealed that LsUVM was 32 per cent dissimilar with wild type of *L. saksenae,* while LlUV1 was 31.5 per cent dissimilar to wild type of *L. lecanii.* The use of REP primers to differentiate mutants was earlier attempted by Abbasi *et al.* (2016) in *Trichoderma harzianum.* Rifai where gamma induced mutants were differentiated using REP-PCR primer, rep1R-I, where it produced more polymorphic bands and detected more loci than other rep primers. Soufi *et al.* (2021) revealed that UPGMA analysis of REP - PCR fingerprints in *Trichoderma aureoviride,* gamma induced mutants varied 70 per cent from the wild type of culture.

One more mutant of *L. lecanii* (LlUV2) obtained at 20 min of UVC exposure exhibited 37.5 per cent molecular variation than wildtype, but did not show any improvement in tolerance or virulence. Therefore, it is speculated that molecular study combined with tolerance studies are needed to ascertain development of UV tolerant mutant.

The results of this study confirms that the REP primers can be used to study the genetic variability between the mutants of entomopathogens and its wild type.

5.5 PATHOGENICITY OF IMPROVED STRAINS

Directed selection for improvement of a particular trait may inadvertently lead to selection of an inferior level of another trait (Gaugler, 1987, De Crecy *et al.*, 2009).

To ascertain the impact of artificial selection on virulence, pathogenicity assay of fungicide tolerant strains was done against *Leptocorisa oratorius* Fab. The results revealed that artificial selection for carbendazim tolerance did not cause any negative impact on virulence of *L. saksenae,* as the mortality recorded was 100 per cent with the selected strain and mother culture. In hexaconazole tolerant strain virulence was found to be decreased as the mortality recorded was 66.67 per cent which is significantly less than that observed with mother culture treatment (100 per cent mortality). The mortality of *L. oratorius* was higher in selected strain compared to non-selected culture in the presence of respective fungicide.

The non-impact of artificial selection on carbendazim tolerance strain virulence was supported by finding of Shapiro -Ilan *et al.* (2002), where artificial selection did not cause any negative impact of virulence of *B. bassiana* when tested on pecan nut weevil, *Curculio caryae* (Horn). Whereas, the result pertaining to decrease in virulence in hexaconazole tolerant strain was supported by Shapiro -Ilan *et al.* (2011) in which a decrease in virulence was observed in *B. bassiana* and *M. brunneum* selected strains when tested on *Galleria mellonella* L. compared to their wild types.

Fungicide tolerant strains developed in this study, through artificial selection is a boon when they are to be applied in combination or sequentially to simultaneously contain pests as well as diseases under field conditions. However, producing such selective populations is still arguable, as there is a possibility that selection may have an adverse effect on other beneficial traits such as virulence. Yet, in the presence of high fungicide concentrations, chosen populations would suppress pests more effectively than populations of the wild type.

Further, Shapiro-Ilan *et al.* (2002, 2011) concluded that selection for fungicide resistance can result in unpredictable impacts either positive or negative, on other traits.

Pathogenicity assay of UV mutants of *L. lecanii* on *Coccidohystrix insolita* Green revealed that LlUVM1 exhibited increased virulence compared to mother culture. LlUVM1 caused 100 per cent mortality on eighth day whereas it was 83.75 per cent in wildtype. The reason for increased virulence of LlUVM1 may be due to increased secondary metabolite production which as suggested by Zhao *et al.* (2016) and Huang *et al.* (2021). They observed an increase in destruxin-A, a secondary metabolite implicated in virulence in *Metarhizium* spp. which led to higher virulence of UV mutants against *P. xylostella.*

Pathogenicity assay of LsUVM on *L. oratorius* carried out as a part of this investigation revealed that there is no significant increase in virulence. This may be due to the quick killing nature of the wild type *L. saksenae* as evidenced by the observation that it took only three days for 100 per cent mortality, unlike other species such as *L. lecanii,* where no mortality was observed (Sankar, 2017) *M. anisopliae* and *B. bassiana* which normally take 7 days to cause 100 per cent mortality (Almeroda and Cruz, 2019).

Investigation on the response of entomopathogens to abiotic stress is very crucial to select a superior isolate or organism which can thrive better under local or prevailing climatic conditions. From the results it can be concluded that the indigenous isolate of *L. saksenae* is comparatively more tolerant to insecticides, temperature and UV exposure than *L. lecanii*. In terms of induction of tolerance, *L. saksenae* responded better to artificial selection for fungicide tolerance and yielded two tolerant strains *viz.*, carbendazim tolerant strain and hexaconazole tolerant strain which performed better in terms of phenotypic characters and virulence than its wild type, in the presence of fungicides.

The UV tolerant mutant developed in both the species were superior to the mother culture under UV irradiance. The findings pave way to the utilization of

the indigenous isolate of *L. saksenae,* its fungicide tolerant strains and the UV mutant of *L. lecanii* in pest management, as they can combat abiotic stress in prevailing climatic conditions.

Summary

6. SUMMARY

The investigation entitled "Tolerance of entomopathogenic fungi *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and *Lecanicillium lecanii* (Zimm.) Zare and Gams to abiotic stress" was conducted at Biocontrol Laboratory for crop pest management, Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, during the period 2017-21. The study aimed in assessing the tolerance level of *L. saksenae* and *L. lecanii* to insecticides, fungicides, temperature and UV radiation and explore the possibilities of developing tolerant strains.

The tolerance study of *L. saksenae* and *L. lecanii* to insecticides and fungicides were carried out based on poisoned food technique where three growth parameters *viz.*, radial growth, sporulation and germination was taken in account. Among the insecticides tested, new generation insecticides such as flubendiamide 39.35% SC, chlorantraniliprole 18.5% SC, imidacloprid 17.8% SL and thiamethoxam 25% WG were least inhibitive where inhibition percentage ranged between three to 23 in growth, 11 to 56 in sporulation and three to 15 in germination, in both the fungi in all the three test doses.

All the old generation insecticides tested caused significant reduction in all the three growth parameters, in both the fungi. Among these dimethoate 30% EC was least inhibitory. Malathion 50% EC and chlorpyrifos 20% EC caused 40 to 60 per cent inhibition in the growth, 20 to 46 per cent in germination and 80 to 90 per cent in sporulation. At all the test doses, quinalphos 25% EC was the most inhibitive with 20 to 32 per cent inhibition in growth, 98 to 99 per cent in sporulation and 36 to 67 per cent in germination.

Compatibility status calculated using BI value revealed that all the four new generation insecticides tested were highly "compatible" at all the three test doses with BI Indices ranging from 83 in flubendiamide 39.35% EC 0.001 %, to 93 in

thiamethoxam 25% WG 0.0025 % for *L. saksenae*. In *L. lecanii* BI values ranged from 71 in imidacloprid 17.8% SL 0.012 % to 95 in thiamethoxam 25% WG 0.0025 %. Dimethoate 30% EC had a high BI value 81 to 89 in *L. saksenae* and 54 to 70 in *L. lecanii.*

All other old generation insecticides such as malathion 50% EC, quinalphos 25% EC and chlorpyrifos 20% EC were "toxic" to *L. saksenae* and *L. lecanii*. The comparison between the BI values revealed that *L. saksenae* had higher BI value than *L. lecanii* in majority of treatments, which proves its superior tolerance to insecticides.

Fungicide tolerance study revealed that azoxystrobin 23% SC was least inhibitive to both the fungi. Based on the BI value (74) it was found to be "compatible" with *L. saksenae* and "moderately toxic" to *L. lecanii,* with a BI value 46. The BI values for copper oxychloride 50% WP were 10 and 44 respectively in *L. saksenae* and *L. lecanii,* indicating that it was "toxic" to *L. saksenae* and "moderately toxic" to *L. lecanii. L. saksenae* grew, sporulated and germinated in carbendazim 50% WP poisoned medium, but *L. lecanii* did not. However, based on BI value it was considered as "toxic" to both the fungi. Mancozeb 75% WP, hexaconazole 5% EC and tebuconazole 25% EC were toxic to both the fungi.

Artificial selection was attempted to induce tolerance to incompatible insecticides and fungicides by subculturing fungi in respective poisoned media at Highest Tolerant Dose (HTD) for ten successive generations. Induction of quinalphos 25% EC tolerance, did not reveal any significant improvement in growth parameters between the "selected" and "non-selected" cultures. Therefore, no improved strain could be developed for quinalphos tolerance in both the fungi.

Induction of fungicide tolerance resulted in two tolerant strains in *L. saksenae* but no improved strain could be developed in *L. lecanii*. Hexaconazole 5% EC tolerant strain of *L. saksenae* was able to tolerate the recommended field dose (0.15

%) which was ten times more than the tolerant level in the non-selected mother culture, where the HTD was 0.015 %. Carbendazim 50% WP tolerant strain exhibited more than 100 per cent increase in growth, sporulation and germination at the recommended dose (0.2%), compared to those in the non-selected culture.

REP-PCR analysis of fungicide tolerant strains of *L. saksenae* revealed the genotypic variation. The variation quantified based on Jaccard's coefficient analysis revealed that carbendazim tolerant strain and hexaconazole tolerant strain were 42 per cent and 38 per cent dissimilar to mother culture. The coefficient analysis also revealed that selected and relaxed cultures were 100 per cent similar to each other which proves the genetic stability of improved strains even after the removal of selection pressure.

Temperature tolerance study carried out at varying regimes of 30, 32, 34, 36, 38 and 40 °C (8 h exposure) revealed that, increase in temperature significantly affected the growth parameters of both the fungi. At 30, 32, 34 and 36 °C a relative growth of 74.79, 63.84, 28.52 and 16 per cent was noted in *L. saksenae* which was significantly higher than that in *L. lecanii* where it was 66.32, 33.67, 23.65 per cent and 0 respectively. Growth of *L. saksenae* terminated at 40 °C, while in *L. lecanii* it was terminated at 36 °C.

In *L. saksenae*, the spore load determined was 10^7spores mL^{-1} at 30, 32 and 34 °C. In *L. lecanii* there was exponential reduction with every two degrees rise in temperature. It was 10^7 spores mL^{-1} in 30 °C; 10^6 spores mL^{-1} in 32 °C and 10^5 spores mL^{-1} in 34 °C. Germination of 85 to 100 per cent was observed at 30, 32 34 and 36 °C in *L. saksenae*, whereas *L. lecanii* exhibited only 13 to 77 per cent germination. Even at 40 °C, *L. saksenae* spores were able to germinate while in *L. lecanii* germination was completely inhibited at this temperature.

Attempts to induce temperature tolerance by sub culturing the fungi at Highest Tolerant Temperature for 10 generations did not yield any tolerant strain.

UV tolerance study conducted with two irradiances wave length *viz.*, UVA (365 nm) and UVB (290 to 315 nm) for short exposure (10, 20, 30 min) and longer exposure (1h, 2h, 3h and 4h). In UVA short exposures there was 13 to 25 per cent reduction in growth, whereas in long exposures the reduction was 26 to 75 per cent reduction. Both, short and long exposures did not affect the sporulation in both the fungi, adversely. It was 10^7 spores mL^{-1} in short and long exposures as well as in the control. In shorter exposures, germination was higher (31 to 73 per cent) in *L. lecanii* compared to *L. saksenae* (25 to 44 per cent). On the other hand, in longer exposures, germination rate was significantly more in *L. saksenae* (15 to18 per cent) compared to *L. lecanii* (13 to 15 per cent).

In UVB irradiance, growth reduction ranged from 12 to 32 per cent in short exposures and 47 to 85 per cent in long exposure in both the fungi. Both, short and long exposures did not affect the sporulation in both the fungi, adversely. It was 10^7 spores mL^{-1} in short and long exposures as well as in the control. Germination of *L. lecanii* was higher (38 to 51 per cent) in shorter exposures compared to *L. saksenae* (21 to 39 per cent). In longer exposures, germination rate was more in *L. saksenae* (13 to 16 per cent) compared to *L. lecanii* (9 to 12 per cent). Comparative analysis between the two species revealed that *L. saksenae* exhibited more growth (5 to 10 per cent) and germination (two to five per cent) at longer exposures than *L. lecanii.*

UV mutagenesis using UVC irradiance resulted in two UV mutants one each in *L. saksenae* and *L. lecanii. L. saksenae* mutant was named as LsUVM and *L. lecanii* mutant, LlUVM1. Both the mutants were superior in terms of growth parameters when tested under UVA and UVB irradiance compared to their mother cultures. The REP-PCR analysis revealed that UV mutants LsUVM and LlUVM1 varied genotypically from their mother cultures. Based on Jaccard's coefficient there was 32 per cent dissimilarity with their respective mother cultures.

Bioassay carried out to study the effect of artificial selection on the pathogenicity of fungicide tolerant strains of *L. saksenae* on rice bug *Leptocorisa oratorius* Fab. revealed that spore suspensions of carbendazim tolerant and hexaconazole tolerant strains @ 10^{7} spores mL^{-1} in the presence of respective fungicide, exhibited higher virulence than the mother culture. Mortality caused by the tank mix formulation of carbendazim tolerant strain + 0.2% carbendazim 50% WP was 86.67 per cent, while its mother culture resulted in only 36.67 per cent mortality. Hexaconazole tolerant strain caused 60 per cent mortality while the mother culture did not cause mortality when applied as tank mix formulation with hexaconazole 5% EC 0.15 %.

Pathogenicity assay of LsUVM, revealed that there was no significant difference in mortality of *L. oratorius* between the mother culture and the mutant. LlUVM1 exhibited higher virulence on the brinjal mealybug, *Coccidohystrix insolita* Green . LlUVM1 caused 100 per cent mortality on the eighth day, while the mother culture of *L. lecanii* caused only 83.75 per cent mortality.

Therefore, the study concludes that both the fungi were compatible with new generation insecticides and one old generation insecticide *i.e.,* dimethoate 30% EC. The indigenous isolate *L. saksenae* is comparatively more tolerant to insecticide, temperature and UV exposure compared to *L. lecanii.* Strains developed for carbendazim and hexaconazole tolerance exhibited difference in phenotypic characters and molecular characters. The UV tolerant strains developed in both the species were superior to the mother culture in terms of growth and germination, under UV irradiance. While UV tolerant strain of *L. lecanii* exhibited higher virulence on *C. insolita,* than the mother culture. The findings pave way to the utilization of the indigenous isolate of *L. saksenae* and its fungicide tolerant strain and the UV mutant of *L. lecanii* in pest management as they can combat abiotic stress in prevailing climatic conditions.

Salient findings

- ✓ *L.saksenae* and *L. lecanii* are compatible with all new generation insecticides tested.
- ✓ Old generation insecticides except dimethoate were toxic to *L. saksenae* and *L. lecanii.*
- ✓ All the fungicides were toxic to both the fungi except azoxystrobin 23% SC which was non-toxic to *L. saksenae*
- ✓ Two fungicide tolerant strains, carbendazim tolerant and hexaconazole tolerant strains could be developed which varied genetically from the mother culture.
- ✓ *L. saksenae* exhibited higher thermotolerance up to 36 °C than *L. lecanii* up to 34°C.
- ✓ *L. saksenae* exhibited higher tolerance to UV irradiance than *L. lecanii.*
- ✓ Two UV tolerant mutant could be developed, one each of both the species.
- ✓ Fungicide tolerant strain exhibited higher virulence in presence of fungicides.
- ✓ UV mutant of *L. lecanii* (LIUVM1) expressed higher virulence on mealybug.

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TOLERANCE OF ENTOMOPATHOGENIC FUNGI *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno AND *Lecanicillium lecanii* (Zimm.) Zare and Gams TO ABIOTIC STRESS

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ABSTRACT OF THE THESIS

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ABSTRACT

The study entitled "Tolerance of entomopathogenic fungi *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno and *Lecanicillium lecanii* (Zimm.) Zare and Gams to abiotic stress" was conducted at Department of Agricultural Entomology, College of Agriculture, Vellayani, Thiruvananthapuram, during the period 2017-22. The objective of study was to assess the tolerance level of *L. saksenae* and *L. lecanii* to insecticides, fungicides, temperature and UV radiation and explore the possibilities of developing tolerant strains.

Results of the experiment to study the tolerance of *L. saksenae* and *L. lecanii* to insecticides revealed that the new generation insecticides *viz.,* flubendiamide 39.35% SC, chlorantraniliprole 18.5% SC, imidacloprid 17.8% SL and thiamethoxam 25% WG were compatible with both the fungi in half dose, recommended dose and double the recommended doses. Among the old generation insecticides, only dimethoate 30% EC was found to be compatible at half and recommended doses. Malathion 50% EC, quinalphos 25% EC and chlorpyrifos 20% EC were found to be toxic to both the fungi at all the test doses. Among all the insecticides quinalphos 25% EC was found to be highly toxic with a Biological Index (BI) of 14 to 22.

Among the fungicides tested, azoxystrobin 23% SC was found to be compatible with *L. saksenae* and it was moderately toxic to *L. lecanii*. Copper oxychloride 50% WP was found to be moderately toxic to *L. lecanii* and toxic to *L. saksenae*. Carbendazim 50 % WP, mancozeb 75% WP, hexaconazole 5% EC and tebuconazole 25 % EC were found to be toxic to both the fungi based on BI values.

Artificial selection to induce tolerance to incompatible insecticides and fungicides was attempted by subculturing fungi in respective poisoned media for ten successive generations. No improved strain could be developed for quinalphos tolerance in both the fungi. Induction of fungicide tolerance resulted in two tolerant

strains in *L. saksenae* but no improved strain could be developed in *L. lecanii*. Hexaconazole tolerant strain of *L. saksenae* was able to tolerate the recommended field dose (0.15 %). Carbendazim tolerant strain exhibited increase in growth, sporulation and germination at the recommended dose (0.2%) compared to those in the non-selected culture. REP-PCR data revealed the genotypic variation of the strains. The variation quantified based on Jaccard's coefficient analysis revealed that carbendazim tolerant strain and hexaconazole tolerant strain were 42 per cent and 38 per cent dissimilar from mother culture.

Temperature tolerance study carried out at varying regimes of 30, 32, 34, 36, 38 and 40 °C (8h exposure) revealed that increase in temperature significantly affected the growth parameters of both the fungi. Growth of *L. lecanii* and *L. saksenae* was completely inhibited at 36 and 40 °C, respectively. In terms of sporulation, no exponential reduction (>10^7 spores mL^{-1}) was observed till 34 ° C in *L. saksenae,* while in *L. lecanii* it was reduced to 10^5 spores mL^{-1} and it was terminated at 36 °C. Comparative tolerance between the species revealed that *L. saksenae* performed better than *L. lecanii*. No significant reduction (<15 per cent) in terms of germination was observed in *L. saksenae* till 36 °C, while in *L. lecanii* more than 70 per cent reduction was observed at 34 °C. *L. saksenae* spores were able to germinate till 40 °C while in *L. lecanii* germination was completely inhibited at this temperature. Attempts to induce temperature tolerance by subculturing the fungi at highest tolerant temperature for ten generations did not yield any tolerant strain.

UV tolerance study conducted with two irradiances wave length *viz.,* UVA - 365 nm and UVB - 290 to 315 nm for 10, 20, 30 min, 1h, 2h, 3h and 4h exposure durations, revealed that the growth of both the fungi was not significantly affected when exposed for shorter durations (10, 20 and 30 min), whereas when exposed for longer durations (1, 2, 3 and 4h), there was significant reduction in growth. In both the wave lengths and across the exposure durations, *L. saksenae* exhibited higher colony growth than *L. lecanii.* In UVA irradiance, the growth reduction ranged

between 13 to 75 per cent in *L. saksenae* and it was 23 to 81 per cent in *L. lecanii*. In UVB irradiance, colony reduction was between 12 to 85 per cent in *L. saksenae* and 31to 89 per cent in *L. lecanii.* In terms of sporulation, no exponential reduction was observed in both the fungi. There was germination inhibition in both the fungi exposed to UVA and UVB irradiance. In shorter exposures, *L. lecanii* exhibited higher germination (30 to 51 per cent) while in longer exposures *L. saksenae* exhibited higher germination (13 to 16 per cent).

UV mutagenesis resulted in two UV mutants one each in *L. saksenae* and *L. lecanii. L. lecanii* mutant was named as LlUVM1 and *L. saksenae* mutant was named as LsUVM. Both the mutants were superior in terms of growth parameters when tested under UVA and UVB irradiance when compared to their mother culture. The REP-PCR analysis revealed that UV mutants LsUVM and LlUVM1 varied genotypically from the mother culture. Based on Jaccard's coefficient there was 32 per cent dissimilarity with their respective mother cultures.

Bioassay carried out to study the effect of artificial selection on the pathogenicity of fungicide tolerant strains of *L. saksenae* on rice bug *Leptocorisa oratorius* Fab. revealed that spore suspensions of carbendazim and hexaconazole tolerant strains of *L. saksenae* (10^7 spores mL $^{-1}$) in the presence of respective fungicide, exhibited higher virulence than the mother culture. Mortality caused by the tank mix formulation of carbendazim tolerant strain + 0.2 % carbendazim 50 % WP caused 86.67 per cent mortality, while its mother culture resulted in 36.67 per cent mortality. Hexaconazole tolerant strain caused 60 per cent mortality while the mother culture did not cause mortality with tank mix of hexaconazole 5 % EC 0.15 %.

Pathogenicity assay of *L. saksenae* UV mutant revealed that there was no significant difference in mortality of *L. oratorius* between the mother culture and the mutant. In *L. lecanii* the UV mutant (LlUVM1) exhibited higher virulence on the

brinjal mealybug, *Coccidohystrix insolita* Green. LlUVM1 caused 100 per cent mortality on the eighth day, while the mother culture of *L. lecanii* caused 83.75 per cent mortality.

Therefore, the study concludes that the indigenous isolate *L. saksenae* is comparatively more tolerant to insecticides, temperature and UV exposure than *L. lecanii*. Strains of *L. saksenae* developed for carbendazim and hexaconazole tolerance performed better in terms of phenotypic characters. Genotypically, they expressed variation of 42 and 38 per cent respectively from mother culture. The UV tolerant strain developed in both the species were superior to the mother culture under UV irradiance. The findings pave way to the utilization of the indigenous isolate of *L. saksenae* and its fungicide tolerant mutants and the UV mutant of *L. lecanii* in pest management as they can combat abiotic stress in prevailing climatic conditions.

സംഗ്രഹം

വെള്ളായണി കാർഷിക കോളേജിലെ എന്റോമോളജി വിഭാഗത്തിൽ 2017 - 2022 കാലയളവിൽ നടന്ന പി എച്ച്. ഡി. ഗവേഷണം, "മിത്രകുമിളുകളായ ലേകാനിസിലിയം സക്സെനെ (*L.saksenae)*, ലേകാനിസിലിയം ലേകാനി (*L. lecanii*) എന്നിവയുടെ അജൈവ സമ്മർദ്ദ ഘടകങ്ങളോടുള്ള സഹിഷ്ണുത" യെ കുറിച്ചായിരുന്നു.

L. saksenae ITCC 7714 എന്ന മിത്രകുമിൾ വെള്ളായണിയിലെ മണ്ണിൽ നിന്നും ഉരുത്തിരിച്ചു എടുത്തതും *L. lecanii* NBAIR ഇനവുമാണ്. ആദ്യത്തെ പരീക്ഷണത്തിൽ നിന്നും തെളിയിക്കപ്പെട്ടത്, നൂതന കീട നാശിനികളായ ഫ്ലുബണ്ടയാമൈഡ് 39.35 % SC ക്ലോറാൻട്രാലിനിപ്രോൾ 18.5 % SC, ഇമിടാക്ലോപ്രൈഡ് 17.8% SL, തയാമേതോക്സാം 25 % WG എന്നിവ മിത്രകുമിളുകളുടെ വളർച്ചയെ സാരമായി ബാധികുന്നില്ല എന്നാണ്. പഴയ തലമുറയിലെ രാസ കീടനാശിനികളായ ഡൈമെത്തോവെറ്റ് 30 % EC, താരതമ്യേനെ മിത്രകുമിളുകളുമായി ചേർന്ന് പോകുമെങ്കിലും മാലത്തിയോൺ 50% EC കിനാൽഫോസ് 25 % EC ക്ലോർപൈറിഫോസ് 20% EC എന്നിവ മിത്രകുമിളുകളുടെ വളർച്ചയെ പ്രതികൂലമായി ബാധിച്ചിരുന്നു.

നൂതന രാസകുമിൾ നാശിനികളിൽ, അസോക്സിസ്ട്രോബിൻ 23 % SC, *L.saksenae* യുടെ വളർച്ചയെ പ്രതികൂലമായി ബാധിച്ചിരുന്നില്ല. എങ്കിലും *L.lecanii* ക്ക് ദോഷകരമായിരുന്നു. പഴയ തലമുറയിലെ കുമിൾ നാശിനികളിൽ കോപ്പർ ഓക്സിക്ലോറൈഡ് മിതമായ ദോഷവും കാർബെൻഡാസിം മാകോസെബ് എന്നിവ ദോഷകരവുമാണെന്ന് കണ്ടു. പുതിയ കുമിൾ നാശിനികളായ ടെബുകൊണസോൾ ഹെക്സകോണസോൾ എന്നിവ ഈ മിത്രകുമിളുകളുടെ വളർച്ചയെ സാരമായി ബാധിച്ചു.

പ്രതികൂലമായി കണ്ടെത്തിയ കീടനാശിനികളിൽ നിന്ന് കിനാൽഫോസും കുമിൾ നാശിനികളിൽ നിന്ന് കാർബെൻഡാസിം ഹെക്സകോണസോൾ എന്നിവയും തിരഞ്ഞെടുത്ത് ഇവക്ക് ടോളറൻറ് ആയിട്ടുള്ള പുതിയ സ്ട്രെയിനുകൾ വികസിപ്പിച്ച് എടുക്കുക എന്നിവ ആയിരുന്നു തുടർന്നുള്ള ഉദ്ദേശം. ഇതിന്റെ ഫലമായി *L.saksenae* യുടെ ഹെക്സകോണസോൾ ടോളറൻറ് സ്ട്രെയിനും കാർബെൻഡാസിം ടോളറൻറ് സ്ട്രെയിനും ഉരുത്തിരിച്ച് എടുക്കാൻ കഴിഞ്ഞു. REP-PCR ഡാറ്റ സ്ട്രെയിനുകളുടെ ജനിതക വ്യതിയാനം വെളിപ്പെടുത്തി. കാർബൻഡാസിം ടോളറന്റ് സ്ട്രെയിനും ഹെക്സകോണസോൾ ടോളറന്റ് സ്ട്രെയ്നും 42 ശതമാനവും 38 ശതമാനവും മാതൃ കൽച്ചറിൽ നിന്ന് വ്യത്യസ്തമാണെന്ന് ജാക്കാർഡിന്റെ കോഫിഷ്യന്റ് വിശകലനത്തെ അടിസ്ഥാനമാക്കി കണക്കാക്കിയ വ്യത്യാസം വെളിപ്പെടുത്തി.

ഉയർന്ന താപനിലയിൽ പ്രവർത്തന ശേഷി നഷ്ടപ്പെടാതെ ഒരു ടെംപെറേച്ചർ ടോളറൻറ് സ്ട്രയിനും വികസിപ്പിച്ചെടുക്കാനുള്ള ഉദ്ധ്യമത്തിന്റെ ഫലമായി *L. saksenae* ക്കു *L. lecanii* യെക്കാൾ സഹിഷ്ണുത കൂടുതലാണെന്നു കണ്ടെത്തി. 40 °C വരെ *L. saksenae* വിത്തുകൾക്ക് മുളയ്ക്കാൻ കഴിഞ്ഞിരുന്നു, *L. lecanii* ഈ താപനിലയിൽ മുളയ്ക്കുന്നത് പൂർണ്ണമായും തടസപ്പെട്ടു.

UV കിരണങ്ങളാൽ പ്രവർത്തന ശേഷി നഷ്ടപ്പെടാത്ത മേൽത്തരം സ്ട്രെയിൻ ഉരുത്തിരിച്ചെടുക്കുക എന്ന ഉദ്ധ്യമത്തിന്റെ ഫലമായി 2 UV മ്യുട്ടന്റുകൾ കണ്ടെത്താൻ കഴിഞ്ഞു. *L. saksenae, L. lecanii* എന്നിവയുടെ ഓരോന്ന് വീതം. UVA - 365 nm, UVB - 290 - 315 nm എന്നിങ്ങനെ രണ്ട് വികിരണ തരംഗദൈർഘ്യം ഉപയോഗിച്ച് നടത്തിയ യുവി ടോളറൻസ് പഠനത്തിൽ 10, 20, 30 മിനിറ്റ്, 1h, 2h, 3h, 4h ദൈർഘ്യത്തിൽ, രണ്ട് ഫംഗസുകളുടെയും വളർച്ച രേഖപ്പെടുത്തി. കുറഞ്ഞ

സമയത്തേക്ക് (10, 20, 30 മിനിറ്റ്) കാര്യമായി ബാധിക്കില്ല, അതേസമയം കൂടുതൽ നേരത്തെ (1, 2, 3, 4 മണിക്കൂർ) എക്സ്പോഷർ വളർച്ചയിൽ ഗണ്യമായ കുറവുണ്ടാക്കി.

നെല്ലിലെ ചാഴിയിൽ കുമിൾനാശിനി സഹിഷ്ണുതയുള്ള *L. saksenae* സ്ട്രെയിനുകളുടെ ബയോഅസെ നടത്തി. കാർബൻഡാസിം, ഹെക്സാക്കോനാസോൾ സഹിഷ്ണുതയുള്ള *L. saksenae* (10^{7} സ്പോർസ് എം.എൽ$^{-1}$) സ്പോർ സസ്പെൻഷനുകൾ മാതൃ കൽച്ചറിനേക്കാൾ ഉയർന്ന പ്രവർത്തനശേഷി പ്രകടമാക്കി. ഹെക്സക്കോണസോൾ ടോളറന്റ് സ്ട്രെയിൻ ഉപയോഗിച്ചപ്പോൾ 60 ശതമാനം ചാഴികളൾ ചാവുകയും, അതേസമയം മാതൃ കൽച്ചർ + ഹെക്സകോണസോൾ 0.15% ടാങ്ക് മിശ്രിതം ഉപയോഗിച്ചപ്പോൾ ഒരു ചാഴിയും ചത്തതായി കണ്ടെത്തിയില്ല .

L. saksenae UV മ്യൂട്ടന്റും മാതൃ കൽച്ചറും തമ്മിൽ കാര്യമായ വ്യത്യാസമില്ലെന്ന് വെളിപ്പെടുത്തി. *L. lecanii* ൽ UV മ്യൂട്ടന്റ് (LIUVM1) വഴുതന മീലിബഗ് എതിരെ ഫലപ്രദം ആണെന്ന് കണ്ടെത്തി. LIUVM1 എട്ടാം ദിവസം 100 ശതമാനം മീലിബഗ്നെയും നശിപ്പിച്ചു.

L. saksenae, L. lecanii നേക്കാൾ കീടനാശിനികൾ, താപനില, UV എക്സ്പോഷർ എന്നിവയോട് താരതമ്യേന കൂടുതൽ സഹിഷ്ണുത പുലർത്തുന്നുവെന്ന് പഠനം നിഗമനം ചെയ്യുന്നു. കാർബൻഡാസിമിനും ഹെക്സകോണസോൾ ടോളറൻസിനും വേണ്ടി വികസിപ്പിച്ചെടുത്ത *L. saksenae* സ്ട്രെയിനുകൾ മികച്ച പ്രകടനം കാഴ്ചവച്ചു. ജനിതകപരമായി, അവ മാതൃ കൽച്ചറിൽ നിന്ന് യഥാക്രമം 38-42 % വ്യത്യാസം പ്രകടിപ്പിച്ചു. രണ്ട് അൾട്രാവയലറ്റ് ടോളറന്റ് സ്ട്രെയിനുകളും മാതൃ കൽച്ചറിനേക്കാൾ മികച്ചതായിരുന്നു.

നിലവിലുള്ള കാലാവസ്ഥാ സാഹചര്യങ്ങളിൽ മികച്ചതായിരുന്നു അജൈവ സമ്മർദ്ദത്തെ ചെറുക്കാൻ കഴിയുന്നതിനാൽ, എൽ.സക്സെനേയും അതിന്റെ കുമിൾനാശിനി സഹിഷ്ണുതയുള്ള മ്യൂട്ടന്റുകളും എൽ.ലെക്കാനിയുടെ യുവി മ്യൂട്ടന്റും കീടനിയന്ത്രണത്തിൽ ഉപയോഗപ്പെടുത്തുന്നതിന് ഈ കണ്ടെത്തലുകൾ വഴിയൊരുക്കുന്നു.