A STUDY ON THE POTENTIAL OF NATIVE ENTOMOPATHOGENIC FUNGI AGAINST MELON/COTTON APHID, *APHIS GOSSYPII* GLOVER (HOMOPTERA: APHIDIDAE)

BY

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DEDICATIONS

To my father and mother: TESFAYE DEGEFU AND BELAYNESH DEMEK, to my wife FREHIWOT AKLILU and my child HENOK DAWIT, this work is further dedicated to W/r SINTAYEU BELETE.

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LIST OF SYMBOLS AND ABRIVATION

APPRC	Ambo Plant Protection Research Center
CM	Complete Medium
DLCO-EA	Desert Locust Control Organization for Eastern Africa
EIAR	Ethiopian Institute of Agricultural Research
ICIPE	International Center of Insect Physiology and Ecology
LC_{50}	50 % lethal concentration
LT_{50}	50 % lethal time
MEA	Malt extract agar
MPDA	Malt extract peptone dextrose agar
MSFD	Ministry of State Farms Development
OMA	Oat meal agar
PDA	Potato dextrose agar
PDAY	Potato dextrose yeast extract agar
SDA	Sabouraud’s dextrose agar
SDAY	Sabouraud’s dextrose yeast extract agar
WARC	Werer Agricultural Research Center

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ABSTRACT

The effects of five temperature regimes and five artificial culture media on germination, radial growth and sporulation of four Beauveria and two Metarhizium isolates obtained from various ecological zones of Ethiopia were investigated in vitro. The susceptibility of adult Aphis gossypii to four concentration levels (10^5, 10^6, 10^7 and 10^8 conidia ml^{-1}) of these fungal isolates was evaluated with direct spraying bioassay at 20, 25 and 30^0c under laboratory conditions. All experiments were replicated three times using completely randomized design. Germination, radial growth and sporulation of all fungal isolates were slower at 15^0c than 20, 25 and 30^0c. At 35^0c none of the Beauveria isolates were able to germinate after 24 hour post inoculation. A suitable temperature for germination and radial growth was ranged from 20 - 30^0c for all fungal isolates. The maximum rate of growth per day was obtained at 30^0c followed by 25^0c. As regards to artificial culture media, all fungal isolates showed maximum radial growth on OMA. Isolate MM and ICIPE 30 revealed the highest sporulation and fastest germination percentage 60% and 77% after 8 hour on OMA, respectively. The Beauveria isolate, DLCO 105 showed the highest sporulation on SDA and PDA. The maximum rate of growth per day was obtained on OMA with all the fungal isolates. The result of the bioassay showed that at 25 and 30^0c, all tested isolates with 1 x 10^8 conidia ml^{-1} were virulent (73.33 – 93.33% mortality and LT_{50} ranged from 3.82 - 4.96 days) and (82.22 - 100% mortality and LT_{50} ranged from 3.22- 4.01 days) to Aphis gossypii, respectively. The Beauveria isolate DLCO 87 had the lowest LC_{50} value at 20 and 30^0c and DLCO 41 at 25^0c.The results of the present study suggest that the potential use of native entomopathogenic fungi as an alternative method to chemical pesticides may hold promise for the management of Aphis gossypii in Ethiopia.

1. INTRODUCTION

The sucking pest of cotton/melon aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) is a cosmopolitan polyphagous species widely distributed in tropical, sub-tropical and temperate regions. It was first reported as an economic pest in south Carolina in 1854, followed by reports of economic pest status on cotton from much of the South east by 1880 (Slosser *et al*., 1989) and in Texas in 1916 (Paddock, 1919). The importance of *Aphis gossypii* as a cotton pest is increasing throughout the cotton-producing regions of the world (LecLant and Deguine, 1994).

In central Africa, *Aphis gossypii* is the second most important economic cotton pest, following *Helicoverpa armigera* (Hübner). *Aphis gossypii* damage affects the yield of cotton seed as well as the fiber quality. In Ethiopia, on average 22% yield loss is caused by sucking pests, of which aphids alone cause 14% (Tsedeke, 1982; Alemayehu and Ababu, 1985). In the Sudan, an infestation of approximately 300 aphids per leaf reduces growth by 38 - 44%, boll production by 78 - 80% and yield by 60 - 65% (Ripper and George, 1965). If infestation occurs late in the season, the leaves again shade, the boll open prematurely and the lint does not developed fully. If left unchecked, aphids can stunt plant growth, deform and discolor leaves and fruit or cause galls to form on leaves, stems and roots (Hamman, 1985).

Besides this yield loss of aphids' direct impact, it can also cause indirect damage by transmitting several important plant virus diseases and secretion of honeydew. Aphids are important vectors of plant diseases, particularly viruses. The cotton aphid is known to

transmit over 50 plant viruses (Kennedy *et al*., 1962). Many aphid species secrete a sticky substance called 'honeydew' which is similar to sugar water. This energy-rich anal secretion falls on leaves and other parts below the infestation site. A black-colored fungi called 'sooty mold' colonizes honeydew-covered surfaces (Mibey, 1997). As a result, sunlight is unable to reach the leaf surface, restricting photosynthesis that produces the plant sugars and certainly reduces the grade of the seed cotton (Elmer and Brawner, 1975). The contamination of cotton by honeydew causes significant problem in the processing industries (Slosser *et al*., 1989; Ebert and Cartwright, 1997). Honeydew is also feed on by bees, wasps, ants and other insects which may provide protection for the aphids from natural enemies (Howard *et al*., 2001).

Over the years, a very wide range of insecticides have been applied to control cotton aphid. But, there has been a general decline in the effectiveness of several insecticides to control *Aphis gossypii*. Aphids give birth to genetically identical females and survive on a wide host range, giving them efficient mechanisms for developing insecticide resistance (O'Brien *et al*., 1990; Grafton-Cardwell, 1991; Ahmad *et al*., 2003; Nauen and Ebert, 2003; Wang *et al*., 2002; Kerns and Gaylor, 1992). Insecticides can also cause aphid populations to dramatically increase following application as a result of the destruction of natural enemies and failure to control the target pest (Oetting, 1985).

Ethiopia's annual pesticide purchases amount to more than 3000 metric tons, valued at nearly US $ 20 million and insecticides constitute about 71% of the total purchase. Of

which 63.5% are used against cotton pests in the former Ministry of State Farms Development (MSFD) (Tsedeke, 1997).

The over use of specific pesticides year after year against different insect pests result to develop resistance. Environmental residues and chemical air pollution are also a concern, because consumers demand pesticide-free food and safe living environment (Kim *et al*., 2001). Hence, Farmers and growers in the world are under pressure to reduce the use of chemical pesticides so that a combination of methods utilizing selective chemical, non-chemical, and biological control is best for aphid management.

Biological control is an alternative control method (Latge and Papierok, 1988; Hajek and Leger, 1994). This includes natural enemies like predators, parasitoids and pathogens. They are found in the field under natural conditions. Aphids have many natural enemies and several groups have been studied as potential biological control agents for release in greenhouse crops. Predators of aphids sold commercially include ladybird beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), flower flies (Diptera: Syrphidae), and predaceous midges (Diptera: Cecidomyiidae, *Aphidoletes aphidimyza*) (Scopes, 1969; Meadow *et al*., 1985; Gilkeson and Hill, 1987). Parasites of aphids include various parasitic wasps (Hymenoptera: mostly Braconidae, such as *Aphidius* spp.) (Scopes, 1970; Sanderson, 1996; Harizanova and Ekbom, 1997; Hunter, 1997).

But pests can also be managed using specific micro-organisms like entomopathogenic fungi i.e. fungal pathogens which affect insects (Latge and Papierok, 1988; Hajek and Leger, 1994) which are widespread in the natural environment and cause infections in

many pest species. Several species of pathogenic fungi, such as *Beauveria bassiana* and *Verticillium lecanii*, cause diseases in aphids and may be available for use as bioinsecticides (Gardner *et al*., 1984). There is also interest in the commercial development of selected strains of such fungi (Milner, 1997). According to Hajek and Leger (1994) approximately over 700 species of entomopathogenic fungi have been reported of which 10 have been or are currently being developed for insect control.

Microbial pathogens may offer more hope for use as localize biopesticides. Currently, the use of insect pathogen as microbial insecticides are coming into picture with promising results (Charnley, 1991). The entomopathogenic fungi within the deuteromycetes regularly found infective, virtually for all species of insects. Entomopathogenic fungi used for microbial control have a range of desirable characteristics including safety to people, compatibility with other natural enemies, and lack of toxic residues. Because they have contact action, they are good for the control of sap feeding pests, like aphids and whiteflies. In addition to this the relative host specificity of most microbial insect pathogen reduces the fear of its danger against beneficial parasitoids and predators as compared to chemical insecticides. Aphids have been satisfactorily controlled on a variety of greenhouse crops with selected strains of *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Paecilomyces fumosoroseus* (Helyer, 1993; Fournier and Brodeur, 2000). Hence, the aim of this study has the following general and specific objectives in order to evaluate the virulence of native Beauveria and Metarhizium fungal isolates against melon/cotton aphids.

General objectives: to investigate the virulence of Beauveria and Metarhizium isolates against *Aphis gossypii*.

Specific objectives:

1. To evaluate the effect of temperature on germination, radial growth and sporulation of Beauveria and Metarhizium isolates *invitro*.
2. To evaluate the effect of solid culture media on germination, radial growth and sporulation of Beauveria and Metarhizium isolates *invitro*.
3. To evaluate the susceptibility of *Aphis gossypii* against different native fungal isolates at preliminary screening stage.
4. To evaluate dose dependent effects of native isolates against the target pest at different temperature regimes *invitro*.

2. LITERATURE REVIEW

2.1 *Aphis gossypii* Glover (Melon /Cotton Aphid)

2.1.1 Diagnosis and host ranges

Aphids, such as the cotton or melon aphid, *Aphis gossypii*, are small, approximately 0.26cm long (Isley, 1946), pear shaped, soft bodied insects. The body is quite variable in colour from light yellow to dark green even within the same aphid colony (Patch, 1925; Wall, 1933; Blackman and Eastop, 1984; Pergande, 1985; Slosser *et al*., 1989; Ebert and Cartwright, 1997). The legs are pale with the tips of the tibiae and tarsi black. Some yellow forms apparently are produced in response to crowding or plant stress. Size also varies considerably according to Wall (1933), this is well correlated with colour. Diagnostic structures found on aphids are siphunculi (cornicles).

Over 250 species of the super family aphidoidea feed on agricultural and horticultural crops throughout the world (Blackman and Eastop, 1984). According to Leonard *et al*. (1971), 350 host plants were recorded. According to Maddox *et al*. (1992) of the 35 different families, aphids attack 64% of the families including 23 composite, 5 polygonaceae and 5 malvaceae. Eastop (1958) reported it on some 15 families in East Africa and on same 60 species in West Africa. Cottier (1953) reported on some 20 plant families. Slosser *et al*. (1989) reported over 60 plant species colonized by cotton aphid. In Europe, Africa and generally in the southern United States, the true *Aphis gossypii* is a polyphagous, anholocyclic species (Stroyan, 1984). However, in America, Japan, China and Korea, *Aphis gossypii* is considered as holocyclic populations over wintering on Rhamnaceae (Kring, 1955; Takada, 1988).

2.1.2 Life cycle of aphid

Most aphids reproduce sexually and develop through gradual metamorphosis. But also reproduce through a process called parthenogenesis. Development to sexual maturity for most aphids depends on weather conditions. Most aphids complete a generation within 5 to 6 days, under optimal conditions in four days (Slosser *et al*., 1989), under warm damp conditions a generation develops within 7 to 10 days (Frohlich and Rodewald, 1970). The cotton aphid may complete a maximum of 51 generations a year (Frohlich and Rodewald, 1970; Munro, 1987). Each adult producing in excess of 80 offspring (Slosser *et al*., 1989). In the summer months, new generations can occur every five days (Anononymus, 1960). The average length of life of an adult is about one month (Little, 1972; Munro, 1987). The rate of reproduction increases as the temperature rises from 17 - 28^{o}c (Isely, 1946), but the rate of reproduction (2.7 offspring per day) occurs at 19-20^{0}c. Host alteration is common in aphids. This process involves a primary host used for sexual reproduction, and a secondary host is utilized for asexual reproduction (Blackman and Eastop, 1984).

2.1.3 Damage and economic importance

Aphids directly feed by sucking sap from their hosts. The undersides of leaves are preferred, other leaf surfaces and flower buds are their next choice but the entire host may be covered when populations are large. *Aphis gossypii* does not cause any apparent mechanical damage to tissues by laceration or biochemical damage by spread of saliva or plasmolysis as with other aphids or mirids and jassids (Johnson, 1934). Aphids also cause indirect damage either by sugary exudates (honeydew) and spread of various

phytopathagens causing series disease with specific symptomatology. *Aphis gossypii* is an important vector of over 50-60 plant viruses in a large range of plants (Eastop, 1983; Kennedy *et al*., 1962).

The importance of *Aphis gossypii* as cotton pest is increasing through out the cotton producing regions of the world (Leclant and Deguine, 1994). This is the case in Iraq (Khalid and Al-Zarari, 1983), Turkey, Syria (Broza, 1986) and Romania (Ullah and Paul, 1985). In China damage is caused by aphid at the beginning and in the middle of the cycle during boll formation (Nan *et al*., 1987).

Concerning cotton crops, *Aphis gossypii* together with *Bemisia tabaci*, is a series pest in Sudan and Egypt and in West Africa (Onu, 1989), and Zambia (Javoid *et al*., 1987). *Aphis gossypii* is now considered as the main causes of yield losses in the United State (276 kg ha^{-1}) (Ewing, 1943). According to the report by Price *et al*. (1983) estimated lose was up to 100 kg/ha. Bagwell *et al*. (1991) stated that heavily infested plants are small and less branched than lightly attacked plants and yield loses are caused due to considerable fruit shedding.

2.1.4 Aphid management

A predatory midge, *Aphidoletes aphidimyze*, can feed on more than 60 different species of aphids. It tends to be more effective against the melon aphid. This bright orange colored larvae kills aphid by biting their knee joints, injecting a paralyzing toxin and then

sucking out their body fluids. This midge is most effective in the summer and will go into diapauses during cool, short days (Hunter, 1997; Malais and Ravensburg, 1992).

Chemical control of certain aphid species has become extremely difficult due to resistance to insecticides particularly organophosphate, carbamate and pyrethroid insecticides. Ffrench-Constant *et al*. (1988) suggested that pyrethroids may actually exacerbate a green peach aphid problem by stimulating the products of nymphs. Insecticides can also cause aphid populations to dramatically increase following application as a result of the destruction of natural enemies and failure to control the target pest (Oetting, 1985).

There has been a general decline in the effectiveness of several insecticides to control *Aphis gossypii*, due to the increasing numbers of cases of resistance, especially to carbamates organophosphorus compounds and also pyrethroids on cotton in Africa (Gubran *et al*., 1992), the USA (Grafton-Cardwell, 1991; Kerns and Gaylor, 1992; O'Brien *et al*., 1992), China (Tang *et al*., 1988).

In terms of cultural practices, because of the wide host range of cotton or melon aphid, it makes crop rotation a difficult tactic to implement successfully. Also, crops grown down-wind from infested fields are especially susceptible because aphids are weak fliers and tend to be blown about turbulence. If continuous cropping is implicated in retention of aphid populations then a crop free period is needed. Row covers can be used to inhibit development of aphid populations (Slosser *et al*., 1989; Webb and Linda, 1992).

2.2 Fungal diseases of insects.

Fungi are the predominant pathogens found in insect populations, and are unique in their ability to infect their hosts through the external cuticle; thus they are capable of infecting both soft- and hard-bodied insects (Hajek, 1997). Some fungal isolates have been used as microbiological control agents for many years against a variety of pests, but only few have been used commercially as biopesticides (Ferron, 1985). In a survey from 1983, 12 fungal isolates in various stages of development were listed, some having been used on a large scale (Roberts and Wraight, 1986). Most attempts to exploit insect-killing fungi commercially have focused on the development of selected strains of *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Paecilomyces fumosoroseus* as biopesticides. Aphids have been satisfactorily controlled on a variety of greenhouse crops with these fungi (Helyer, 1993; Fournier and Brodeur, 2000).

Most fungal biopesticides are applied as augmentative control agents, i.e. indigenous species and strains are mass produced and inoculated in their natural habitats. The inoculation leads to a change in the natural host-pathogen equilibrium in favour of the entomopathogen, which then reduces pest populations (Lomer *et al*., 1997). According to Greathead and Prior (1990), the advantage of augmentative control is the possibility of mass production on simple media and the formulation of spores in oil.

2.2.1 Mode of infection

Fungi are unique in their ability to infect their hosts through the external cuticle; thus they are capable of infecting both soft- and hard-bodied insects (Hajek, 1997). Fungi

invade insects by penetrating their cuticle or skin. After attaching to the insect (Bateman *et al*., 1996), conidia penetrate the cuticle with the help of enzymatic degradation and pressure of the germ tube (Ferron, 1981; Starnes *et al*., 1993; Bateman, 1998). The infection may also take place via the respiratory system (Burges and Hussey, 1971). After infection, free flouting, yeast-like hyphal bodies are produced and spread throughout the haemocoel (Flexner *et al*., 1986). *Metarhizium anisopliae* produces destruxins causing paralysis and insects die between three and fourteen days after infection, depending on species and size, on the surface of cadavers, spores are produced (Whitten and Oakeshotte, 1991; Starnes *et al*., 1993) which can subsequently infect other susceptible hosts (Bateman *et al.*, 1996).

2.2.2 Deuteromycotina (Fungi Imperfecti) - Hyphomycetes.

Insect pathogenic fungi are mostly found in the orders of Moniliales (Deuteromycotina: Hyphomycetes syn. Deuteromycetes) and the Entomophthorales (Zygomycotina: Zygomycetes) (Flexner and Belnavis, 1998). Hyphomycetes have been studied for use against a broad range of insect pests including whiteflies, aphids, thrips, termites, grasshoppers and locusts, beetles, and others (Devi, 1994; Feng *et al*., 1994; Ferron *et al*., 1991; Goettel *et al*., 1995; Keller *et al*., 1997; McCoy *et al*., 1988; Milner, 1997; Milner and Prior, 1994; Zimmermann, 1993). Most attempts to exploit insect-killing fungi commercially had focused on the development of selected strains of *Beauveria bassiana*, *Metarhizium anisopliae, Verticillium lecanii* and *Paecilomyces fumosoroseus* as biopesticides.

Compared to other microorganisms, fungi are known to infect a broader range of insects including Lepidoptera, Homoptera, Hymenoptera, Coleoptera and Diptera. Some strains of Beauveria and Metarhizium represent the most frequently used genera (Burges and Hussey, 1971) and cover nearly all orders of insects. As the result, most research on fungi has been directed to *Beauveria* and *Metarhizium* (Greathead and Prior, 1990; Prior, 1992, 1997; Whitten and Oakeshotte, 1991) whose distribution is cosmopolitic (Starnes *et al.*, 1993).

The fungus *Beauveria bassiana* is a common soil born fungus that occurs world wide. MacLeod (1954), recognized two species, *Beauveria bassiana* and *Beauveria brongniartii*, which attack all stages of insects of all groups. The very distinctive and noticeable white mummies of silkworms produced by infection led to its discovery by Augustine Bassi de Loid in 1835 (Steinhause, 1949). The fungus was later named *Beauveria bassiana* in honor of its discoverer (Tanada and Kaya, 1993).

In 1878, research on the green muscardine fungus *Metarhizium anisopliae* (Metsch.) Sorokin started in Russia in order to control beetle larvae in sugarbeet (Greathead and Prior, 1990). *Metarhizium anisopliae* affects a large number of insect groups (Rombach et *al.*, 1986; 1987b). Presently *M. anisopliae* are applied successfully against insect pests of several crops (Ferron, 1981), including pests of rice in the tropics (Rombach *et al.*, 1987a). Over 200 species of insects from seven to nine orders, mainly Lepidoptera and Coleoptera, have since been recorded as hosts of *Beauveria bassiana* and *Metarhizium anisopliae* (Zimmerman, 1993).

Beauveria bassiana was used for control of numerous pests in the People's Republic of China (Feng *et al.*, 1994) and the Colorado potato beetle in the former USSR (Ferron *et al.*, 1991). *Metarhizium anisopliae* has potential against several pest species and is being used commercially in Brazil for control of spittlebugs in sugarcane (Wraight and Roberts, 1987). *Beauveria bassiana* can infect such a wide range of insects by producing spores that are resistant to environmental extremes. Once inside the insect it produces a toxin (beauvericin) that weakens the host's immune system (Feng *et al*., 1994).

Beauveria bassiana is available in commercial strains that better withstand ultraviolet light, temperature and humidity extremes commonly encountered in the field (Tanada and Kaya, 1993). There are several products that contain *B. bassiana*, including Naturalis®, BotaniGard®, and Mycotrol®. Interest in the use of fungi has also been further promoted by the rapid development of insecticide resistance by major greenhouse pests such as thrips, aphids and whiteflies (Prabhaker *et al*., 1985, Broadbent and Pree, 1997).

Beauveria bassiana has shown considerable potential for management of insect pests (Feng *et al*., 1994). However, the successful use of *B. bassiana* in IPM programs might be limited by many abiotic and biotic factors. Abiotic factors affecting *B. bassiana* include temperature, humidity and light. Inglis *et al*. (1997) stated that the efficacy of *B. bassiana* was limited by solar radiation and temperature when used against acridid grasshopper under field conditions. Survival in soil favored by darkness and reductions in both temperature and soil moisture at 8^{o}c and under dry conditions, 90% of the conidia

survive for over 635 day (Clerk and Madellin, 1965; Walstad *et al*., 1970), on silica gel conidia survive up to 36 months of storage at -20^{0}c (Bell and Hamalle, 1974).

Temperature has been considered as an influential factor not only on the germination and mycelial development but also on the speed and quantity of spore production of fungal isolates. Sometimes in *Beauveria bassiana* there are temperature optima for the speed and the quantity of spore production at 30 to 24^{0}c, respectively (Teng, 1962). Fungi are highly dependent on the ambient microclimate where limits of fungal growth range between 5-35^{0}c respectively, with optima between 20-30^{0}c (Flexner *et al.*, 1986; McCoy *et al.*, 1988).

Concerning tolerance at high temperature, a *Metarhizium anisopliae* strain survived at 60^{0}c for 1 h in a suspension (Glaser, 1926); while in another study temperature at 49^{0}c for 10 minute was found to be lethal (Walstad *et al*., 1970). At lower temperature of 8^{0}c the spores of *Beauveria bassiana* and *Metarhizium anisopliae* had 90% and 85% survival after a year, respectively (Clerk and Madellin, 1965).

Penetration of the cuticle by fungal infection is thought to occur by a combination of enzymatic degradation and physical pressure. Evidence for enzymatic degradation includes the disappearance of the wax layer beneath appressoria of *M. anisopliae* on wireworm cuticle (Zacharuk, 1970), and the presence of cuticular holes around germ tubes of *B. bassiana* during penetration of larvae of *Heliothis zea* (Pekrul and Grula, 1979).

The fungus *Beauveria bassiana* invades the larval alimentary canal of *Heliothis zea* and cause starvation and nutrient depletion that may lead to larval death (Cheung and Grula, 1982). Once inside the insect, it produces mycotoxins, such as beauvericin which rapidly debilitate the insect or weakens the host's immune system (Roberts, 1981). However, Champlin and Grula (1979) reported that beauvericin is not produced in sufficient quantities to be involved in the parthenogenesis of *Beauveria bassiana* on *Heliothis zea* larvae. Dead larvae, pupae, and adults originating from Beauveria infected insects are over grown with external mycelium and white conidia within one or two days after death. Conidia are produced on conidiophores that out grow from the surface of an insect.

Some of the major economic insect pests that are susceptible to *Beauveria bassiana* are Whiteflies, Aphids, Grasshoppers, Termites, Colorado potato beetle, Mexican been beetle, Japanese beetle, Boll weevil, Cereal leaf beetle, Bark beetles, Lygus bugs, Chinch bug, Fire ants, European corn borer, Codling moth, *Ostrinia nubilalis*, *Laspeyresia pomonella*, *Popillia japonica*, *Pieries brassicae*, *Blissus leucopterus*, and the European cabbageworm (Tanada and Kaya, 1993). On agar media, conidiogenesis starts after six days, while in liquid culture it takes only 3 - 4 days (Samsinakova, 1966).

Conidia of *M. anisopliae* germinate in response to a range of exogenous carbon and nitrogen sources (Leger *et al*., 1991). There is variation in germination between different strains, which may be related to host species (Leger *et al*., 1994). *Metarhizium anisopliae* var. *anisopliae* isolated from scarabeids germinate at high frequency only in the presence of a crude protein/chitin product. Those isolated from other Coleoptera germinate and

form appressoria in yeast extract (0.0125%) (Leger *et al*., 1994). Isolates from Hemiptera germinate and form appressoria in media containing glucose as the sole carbon source.

The order Hemiptera includes aphids and other sap-sucking bugs. Aphids often become coated in the sugary sap, or honeydew released by their feeding, leading to high concentrations of carbon sources on the cuticle. A successful pathogen on these insects would therefore have to be capable of forming appressoria in the presence of high levels of available carbon. Evidently, nutrient levels are one of the environmental cues enabling the pathogen to 'recognize' if it has landed on an appropriate host cuticle. The level of nutrients on these comparatively thin skinned insects such as Homopteran and Lepidopteran larvae are likely to be higher than those on Coleopterans with hard cuticles, thus leading to different responses (Leger *et al*., 1994).

Cultures of *M. anisopliae* produce destruxins a substances toxic to insects (Suzuki *et al*., 1971) produced by *M. anisopliae* appear to interfere with haemocyte function, especially by suppressing prophenoloxidase activation (Huxham *et al*., 1989). According to Samuels *et al*. (1988), the toxin, destruxins of *M. anisopliae* causes symptoms, principally by paralyzing muscles of caterpillars.

3. MATERIALS AND METHODS

3.1 Study area

The experiments for the present work were conducted at Werer Agricultural Research Center (WARC), which is 278km from Addis Ababa. The Center is found 740m a.s.l. with mean annual temperature and rainfall of 34^0c and 560mm, respectively.

3.2 Fungal culture preparation

The experiments were conducted under Werer laboratory conditions (Appendix 5). The fungal isolates were obtained from Ambo Plant Protection Research Center (APPRC) and DLCO-EA (Appendix 2). All the fungal isolates were maintained on potato dextrose agar (PDA) at 25 ± 0.1^0c in darkness for 12 days. To prepare the suspensions of fungal isolates, the conidia were scraped from the surface of plates with a sterile scalpel and suspended in a sterile, aqueous solution of 0.1% Tween 80. The resulting suspensions were homogenized by thoroughly blending the mixture with a vortexer for two minutes. Each suspension was filtered through sterile muslin to remove mycelia and debris. The concentration of conidia in the filtrate was estimated using hemacytometer under a compound microscope (400x magnification). Suspensions were diluted to give a final concentration of 1 x 10^5, 1 x 10^6, 1 x 10^7, 1 x 10^8 conidia ml^{-1} for the main assay while a single concentration, 1 x 10^8 conidia ml^{-1} was used for the preliminary native isolate screening purpose. The suspensions were placed in refrigerator overnight at 4^0c to prevent germination of conidia before use. The fungal isolates were used in assay after not more than 2 subcultures on PDA to avoid possible loss of virulence associated with continuous culturing (Hajek *et al*., 1990).

3.3 Isolate selection

To select virulent isolates, a total of 59 fungal isolates, 47 Beauveria and 12 Metarhizium were evaluated (Appendix 2). Each fungal isolate was tested with a concentration of 1 x 10^8 conidia ml^{-1} against aphids of approximately the same size and life stage at 25 ± 0.1^0c. The treatments were replicated four times with twenty aphids per plate. Based on speed of kill which was 5 days post inoculation (≥ 75%) and speed of germination after 12 h (≥ 45%) (Appendix 3), six fungal isolates were selected for bioassay study (Appendix 1).

3.4 Study on spore germination

Germination potentials of spores of selected isolates was studied by spreading 0.5ml conidial suspension (from 1 x 10^7 conidia ml^{-1}) in petridishes with PDA and allowed to dry in a laminar flow cabinet before incubation. For each isolate, three plates were inoculated separately at temperature regimes of 15, 20, 25, 30 and 35^0c. A total of 90 plates were inoculated with conidial suspension. Then the plates were sealed with parafilm and incubated at 15, 20, 25, 30, and 35^0c in complete darkness at RH of 60-70%. Germination was assessed after 24 h of incubation by counting 300 spores in each replicate using a compound microscope at 400x magnifications. Spores were considered germinated if the germ tube was at least twice longer than the width of each conidium.

3.5 Study on radial growth

For each isolate used, 0.5ml conidial suspension from 1 x 10^7 conidia ml^{-1} was spread evenly by rotating the plates clock wise gently and incubated in the dark at 25 ± 0.1^0c for

48 h. Plugs with 6mm diameter were cut from these cultures with a flame-sterilized cork borer and placed upside down on the center of fresh PDA in 90 mm Petridishes. One plug per dish was used. Three replicate plates were prepared for each isolate temperature combination and incubated after being sealed with parafilm for 12 days at constant dark conditions at 15, 20, 25, 30 and 35^0c and 60-70% RH on separate days. Radial growth was measured from the predetermined mark with a ruler every day for the duration of the experiment. After 12 days, the plates were transferred to a refrigerator maintained at 4^0c temperature until required.

3.6 Study on sporulation

Sporulation was determined from plates kept in a refrigerator. Conidia were harvested by scraping the surface of the culture with 10 ml of sterile distilled water with 0.1% Tween 80. The spore suspension was then filtered through cheesecloth and diluted (1:10) in sterile water. The suspension was vortexed for 3 minutes to avoid clumping of the spores and 50µl was loaded on to each side of the haemocytometer, and the number of conidia per ml was calculated under a compound microscope (400x) for each plate in the replicate.

3.7 Study on artificial media for fungal germination

Five solid culture media including, Potato dextrose agar (PDA) (40g potato dextrose agar and 1lit sterile distilled water), 2% Malt extract agar (MEA) (20g malt extract, 15g agar and 1lit sterile distilled water), Sabourd dextrose agar (SDA) (10g peptone, 20g dextrose and 15g agar and 1lit sterile distilled water), Oat meal agar (OMA) (72g oat meal agar

and 1lit sterile distilled water) and Malt extract peptone dextrose agar (MPDA) (20g malt extract, 1g peptone, 20g dextrose, 20g agar and 1lit sterile distilled water) were compared for speed of germination of the six fungal isolates. A conidial suspension of 0.5 ml was spread-plated evenly on 15 ml of each of the culture media described above in a Petridish. Each culture medium was replicated three times for each isolate. The dishes were sealed with parafilm and incubated at 26 ± 0.1^0c in the dark and 300 spores were examined for germination after 8, 12, 16, 20 and 24 h.

3.8 Study on artificial media for radial growth

To obtain homogeneous fungal cultures, 0.5 ml spore suspension containing 1 x 10^7 conidia ml^{-1} in sterile distilled with 0.1% Tween 80 was spread evenly on to PDA medium in Petridishes. The Petridishes were sealed with parafilm and incubated at 25 ± 0.1^0c in the dark. After 48 hours mycelial plugs were removed with flame-sterilized cork borer of 6mm diameter and placed upside down on the center of freshly prepared PDA, MEA, SDA, OMA and MPDA. The plates were incubated at 26 ± 0.1^0c in the dark at 60-70% RH. Measurement of radial growth was taken 2, 4, 6, 8, 10, and 12 days post inoculation from predetermined mark using a ruler. Then, plates were transferred to the refrigerator until used.

3.9 Study on artificial media for sporulation

After 12 days, sporulation was assessed from plates in the refrigerator by scraping the conidia carefully with a sterile spatula and poured into test tubes with 10 ml sterile distilled water and 0.1% Tween 80. The spore suspension was then filtered through

cheesecloth and diluted (1:10) in sterile water. The suspension was vortexed for 3 minutes to avoid clumping of the spores and 50µl was loaded on to each side of the haemocytometer, and the number of conidia per ml was calculated under a compound microscope (400x) for each plate in the replicate.

3.10 Bioassay

Younger leaf parts of cotton were cut into 8cm diameter. The surface of each leaf disc was disinfected with 0.1% sodium hypochlorite solution and rinsed twice in sterile distilled water. The leaves were embedded, with only one surface exposed in molten (45-50^{0}c) 2% sterile water agar with no bubbles forming on the undersides of the leaf (Appendix 6). Then water agar embedded dishes were kept at 9^{0}c until used.

3.10.1 Rearing of *Aphis gossypii*

Laboratory colonies of *Aphis gossypii* were individually maintained in a plastic mesh cage with cotton plants. Vigorous wingless adults taken from the colonies were transferred to 2% water agar embedded with Acala SJ_2 cotton leaf discs in Petridishes. Adults were allowed to freely produce nymphs for 48 h at 25 ± 0.1^{0}c with a photoperiod of 16L: 8D h and RH at 65 - 70% under environmental test chamber in order to conform uniformity in post embryonic developmental ages (Appendix 7). Adults were removed after 48 hours and nymphs were allowed for further development until the last ecdysis was completed. Dishes were changed every three days, to avoid any contamination. Following this procedure, the new adults of F_2 generation with age variation of ≤ 2 days were treated.

3.10.2 Inoculation

For the assay, six fungal isolates of four Beauveria and two Metarhizium were used. Fifteen *Aphis gossypii* adults of less than or equal to two days of age variation were gently removed from the colony using hen's feather to avoid cuticular damage and possible infection of the nymph by saprophytic microorganisms through the damaged cuticles. Every fifteen aphids on a leaf disc in Petridish were exposed to a spray of 500µl conidial suspension of 1 x 10^5, 1 x 10^6, 1 x 10^7, 1 x 10^8 conidia ml^{-1} using a hand spray atomizer. The treated aphids were covered with 100 mm diameter filter paper, to avoid water condensation, on top of each Petridish. After exposure to the sprays, dishes were covered with a glass cup. Finally, treated aphids were incubated at 20, 25, and 30 ± 1^0c and a photoperiod of 16L: 8Dh and with RH of 65 -70%. Each fungal concentration for treatment of each fungal isolates included 45 aphids in three Petri dishes sprayed separately. The controls were treated with 0.1% Tween 80 and sterile distilled water. A total of 1,350 aphids were used for all fungal isolates and concentrations including the control per one experiment at each temperature. The experiments were conducted on separate days using two incubators at temperatures variation of ± 0.1^0c. Mortality was monitored daily for 7 days. More than 87% of the cadavers had shown external sporulation as evidence for mycosis. The relative potency of fungal isolates was calculated (Feng and Johnson, 1990). Those with the lowest LC_{50} values as compared to the standard check were selected as virulent isolates.

3.11 Statistical Analysis

Percent germination data obtained after 24 h of incubation for each isolate-temperature and isolate-media combinations at various time intervals were square-root arcsine transformed. Sporulation data were $\log_{10}$ transformed and non transformed data were used for radial growth. Data for percent cumulative mortality after six day of treatment were corrected using Abbott formula (Abbott, 1925). Data for percent cumulative mortality at all time intervals were angular transformed and all data were subjected to two way ANOVA using the general linear model procedure (SAS Institute, 1999-2001). Means were separated using the Tukey's Studentized Range (HSD) test (SAS Institute, 1999-2001). Probit analysis was used to estimate both the LC_{50} and LT_{50} of the isolates (SAS Institute, 1999-2001).

4. RESULTS

4.1 Effect of temperature on germination (%), radial growth and sporulation

4.1.1 Germination

There were significant differences between isolates in mean germination percentage at 15^0c ($F = 67.28$, $df = 5$, $P<0.0001$), 20^0c ($F = 21.63$, $df = 5$, $P<0.0001$), 25^0c ($F = 4.09$, $df = 5$, $P<0.02$), 30^0c ($F = 3.53$, $df = 5$, $P<0.034$), 35^0c ($F = 1502.79$, $df = 5$, $P<0.0001$). Temperature by isolate interaction was significant ($F = 387.65$, $df =20$, $P<0.0001$) (Fig. 1a). Temperatures at 15 and 35^0c were found to be from slightly to highly inhibitory for all tested isolates. Metarhizium isolate (MM) was highly inhibited at 15^0c and germinated only 7% after 24 hour of incubation. While isolate ICIPE 30 showed 30% germination. At 15^0c, germination of Beauveria isolates was between 17 and 31% (Fig. 1a). At 35^0c all Beauveria isolates failed to germinate after 24 hour while the Metarhizium isolates MM and ICIPE 30 germinated at 58 and 89%, respectively. All tested isolates, exhibited 79-100% germination at 20-30^0c after 24 hour of incubation (Fig. 1a). At 25 and 30^0c there was no significant difference in germination between the tested fungal isolates (Fig. 1a).

4.1.2 Radial growth

There were significant differences between isolates in mean radial growth at 15^0c ($F = 31.01$, $df = 5$, $P<0.0001$), 20^0c ($F = 38.54$, $df = 5$, $P<0.0001$), 25^0c ($F = 293.89$, $df = 5$, $P<0.0001$), 30^0c ($F = 799.44$, $df = 5$, $P<0.0001$), 35^0c ($F = 49.00$, $df = 5$, $P<0.0001$). Temperature by isolate interaction was significant ($F=124.93$, $df = 20$, $P<0.0001$) (Fig. 1b). At 15^0c, the radial growth of Beauveria isolates was between 8-18.67mm after 12 days. Isolate DLCO 41 showed the lowest radial growth (8.33mm) and DLCO 87

(18.67mm) at this minimum temperature. The Metarhizium isolates ICIPE 30 and MM showed 15.33 and 18mm after 12 days, respectively at 15^0c. The optimum mycelial growth was obtained at 20-30^0c for all tested isolates. The highest radial growth was recorded at 30^0c (55 mm) by isolate ICIPE 30 followed by MM (47mm). At 35^0c, except isolates MM and ICIPE 30, the optimal growth of which were 1.67 and 2mm, respectively, all the Beauveria isolates failed to grow. Radial growth of Beauveria isolates ranged between (8.33-18.67mm), (18-27.33mm), (18- 41.67mm) and (21-32.67mm) at 15, 20, 25 and 30^0c, respectively. The highest radial growth was recorded at 25^0c for isolate DLCO 87 41.67mm while isolate DLCO 41 showed the lowest 18mm (Fig. 1b).

There were a significant differences in growth rate per day between isolates at 15^0c ($F = 28.84$, $df = 5$, $P<0.0001$), 20^0c ($F = 26.13$, $df = 5$, $P<0.0001$), 25^0c ($F = 288.30$, $df = 5$, $P<0.0001$), 30^0c ($F = 1227.88$, $df = 5$, $P<0.0001$). Temperature by isolate interaction was significant ($F = 98.22$, $df = 15$, $P<0.0001$) (Fig. 2a). The growth rate varied from 0.53mm day $^{-1}$ (DLCO 41) to 1.25mm day^{-1} (MM) and 1.25mm day^{-1} (DLCO 41) to 2.45mm day^{-1} (MM) at 15 and 20^0c, respectively. The growth rate at 25 and 30^0c varied from 1.33mm day^{-1} (DLCO 41) to 3.45mm day^{-1} (MM) and 1.56mm day^{-1} (DLCO 41) to 4.25mm day^{-1} (ICIPE 30), respectively. Beauveria isolates, DLCO 87, DLCO 105 and DLCO 43 showed a reduced growth rate as temperature increased from 25 to 30^0c.

4.1.3 Sporulation

There were a highly significant differences between isolates in sporulation at 15^0c (F = 22.59, df = 5, P<0.0001), 20^0c (F = 152.39, df = 5, P<0.0001), 25^0c (F = 205.01, df = 5, P<0.0001), 30^0c (F = 1426.59, df = 5, P<0.0001). Temperature by isolate interaction was significant (F = 495.61, df = 20, P<0.0001) (Fig. 2b). The lowest sporulation was recorded for the Metarhizium isolate MM and ICIPE 30 at both 15 and 20^0c. At the highest temperature 30^0c a maximum sporulation was obtained from Metarhizium isolates MM (1.57×10^9 conidia ml^{-1}) and ICIPE 30 (1.10×10^9 conidia ml^{-1}). As temperature increased sporulation also increased for Metarhizium isolates. In general, for the Beauveria isolates the optimum temperature for sporulation was found to be from 20-25^0c. However, isolate DLCO 43 gave a sporulation of 4.20×10^8 conidia ml^{-1} at 30^0c. From Beauveria isolates, the lowest sporulation 1.03×10^8 conidia ml^{-1} was obtained from DLCO 87 at 15^0c. At 35^0c, all isolates failed to sporulate.

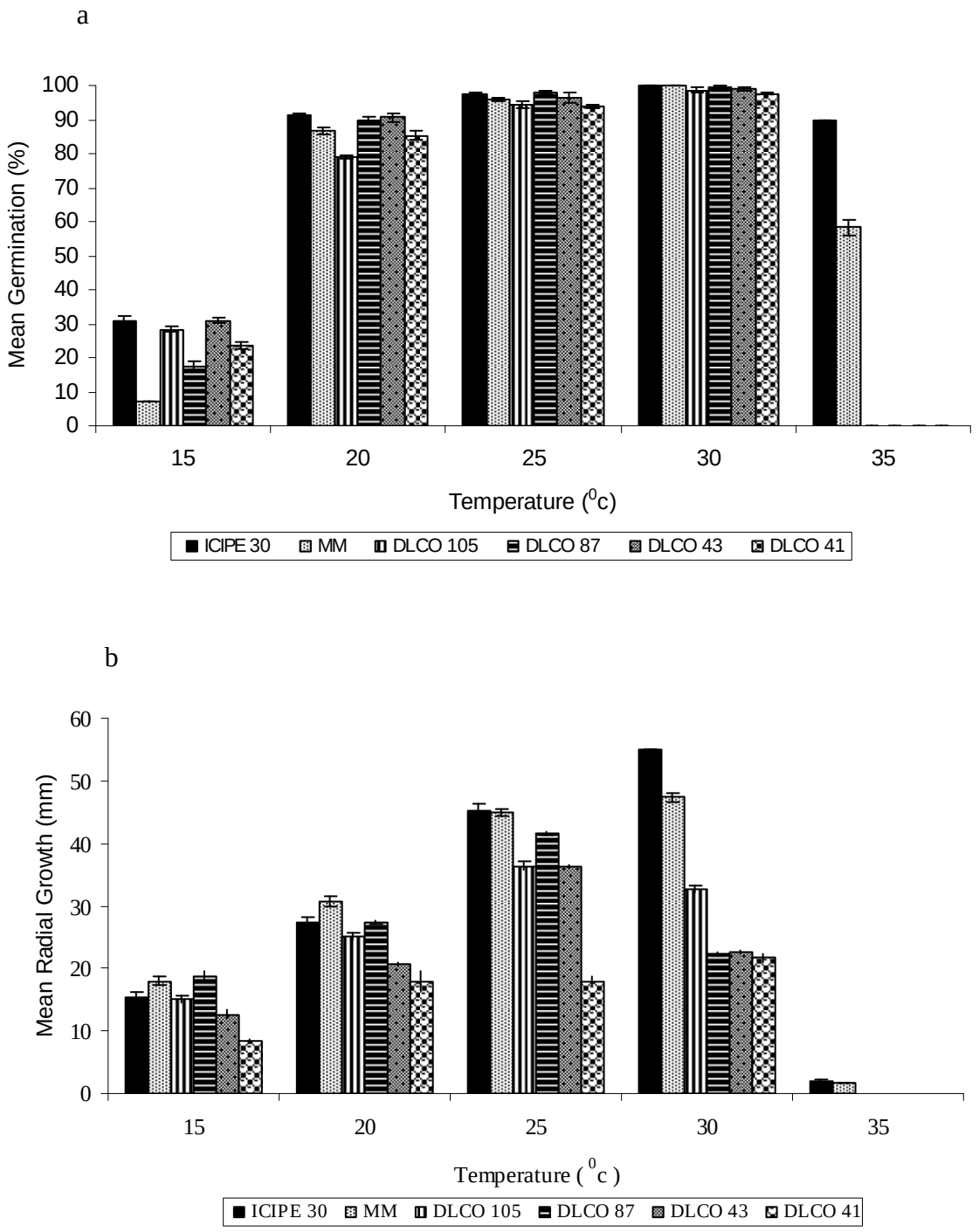


Figure 1. The effect of temperature on (a) the mean germination (%) and (b) radial Growth

ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

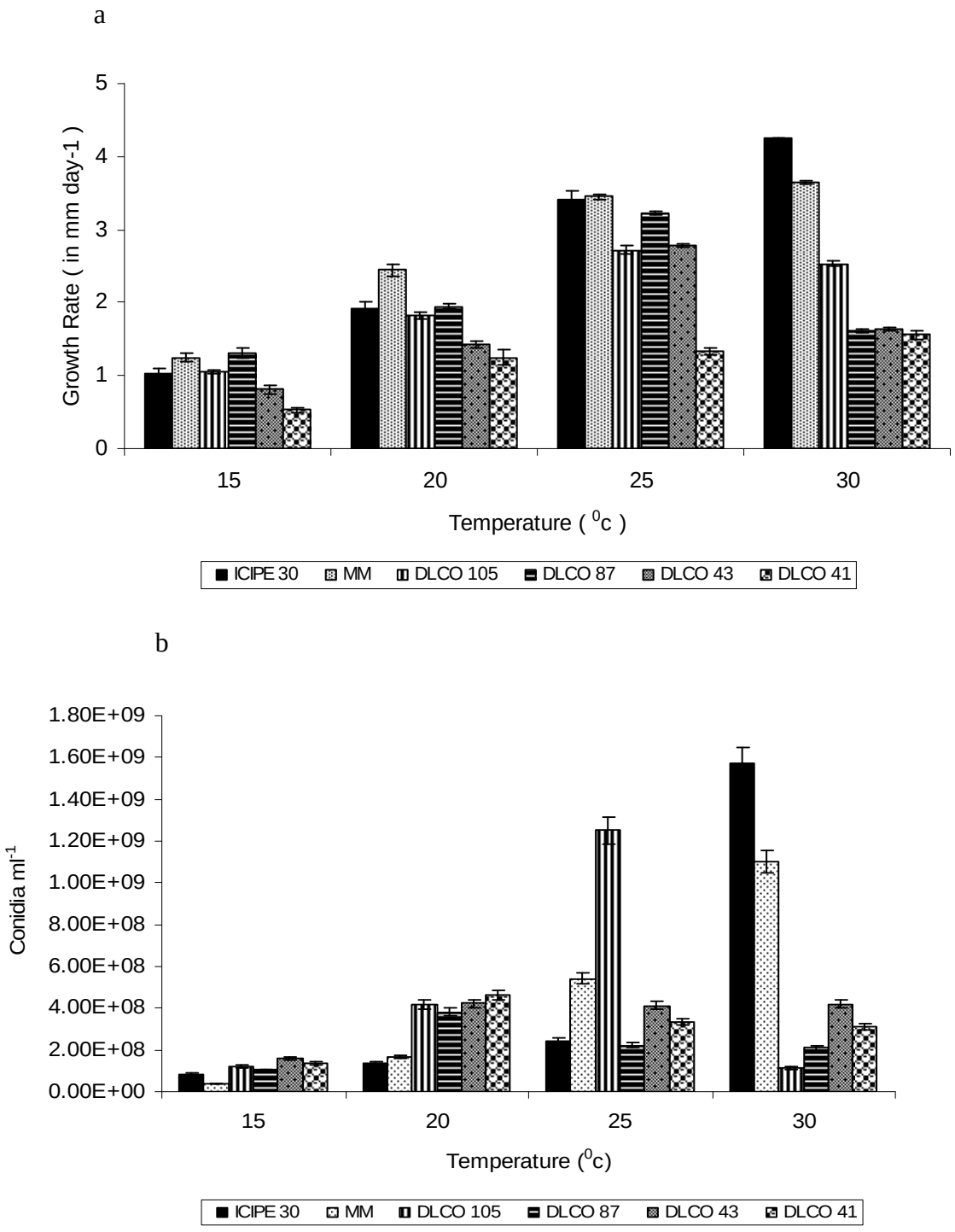


Figure 2. The effect of temperature on (a) the growth rate and (b) Sporulation

ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

4.2 Effect of solid culture media on mean germination (%), radial growth and sporulation

4.2.1 Germination rate

There were a highly significant differences in germination rate between isolates 8h after inoculation on MPDA ($F = 508.27$, $df = 5$, $P<0.0001$), MEA ($F = 177.19$, $df = 5$, $P<0.0001$), SDA ($F = 343.81$, $df = 5$, $P<0.0001$), PDA ($F = 337.97$, $df = 5$, $P<0.0001$). Media by isolate interaction was significant ($F = 21.95$, $df = 15$, $P<0.0001$) (Fig. 3a). The speed of germination was highly variable in the first 8 hour between isolates at each medium. The Metarhizium isolates MM (60%) and ICIPE 30 (70%) germinated much faster than all other isolates on malt extract peptone dextrose agar (MPDA) media 8 hour after inoculation. Among all tested isolates, isolate DLCO 105 gave no germination on SDA and PDA after eight hours. The speed of germination for Beauveria isolates ranged from 2.33 to 20.33% which is much lower than the Metarhizium isolates (48-77%).

There were a highly significant differences in germination rate between isolates 12h after inoculation on MPDA ($F = 108.16$, $df = 5$, $P<0.0001$), MEA ($F = 131.62$, $df = 5$, $P<0.0001$), SDA ($F = 261.55$, $df = 5$, $P<0.0001$), PDA ($F = 142.91$, $df = 5$, $P<0.0001$). Media by isolate interaction was significant ($F = 79.22$, $df = 15$, $P<0.0001$) (Fig. 3b). The lowest germination percentage (23.33%) was recorded for isolate DLCO 105 while 36% germination was recorded for the same isolate on SDA and PDA. Isolate DLCO 87 showed germination percentage of 39.33% and 49.33% on PDA and SDA, respectively. All tested isolates germinated more than 50% at least in one of the media. The highest

germination percentage was obtained on MEA and MPDA by isolate MM (92.67%), ICIPE 30 (92.33% and 91.67%) and DLCO 87 (91% and 90 %)

There were significant differences in germination rate between isolates 16h after inoculation on MPDA ($F = 60.22$, $df = 5$, $P<0.0001$), MEA ($F = 114.22$, $df = 5$, $P<0.0001$), SDA ($F = 157.10$, $df = 5$, $P<0.0001$), PDA ($F = 3.74$, $df = 5$, $P<0.03$). Media by isolate interaction was significant ($F = 46.57$, $df = 15$, $P<0.0001$) (Fig. 4a). Metarhizium isolates, MM and ICIPE 30 showed 97 to 100% and 99.33 to 99.67%, respectively after sixteen hours on all media except on PDA whereas the germination percentage was 89.67% for ICIPE 30 and 92% for MM (Fig. 4a). The lowest germination percentages were recorded on isolate DLCO 105 (48%) and DLCO 87 (57.67%) on SDA.

There were significant differences in germination rate between isolates 20h after inoculation on MPDA ($F = 39.31$, $df = 5$, $P<0.0001$), MEA ($F = 35.42$, $df = 5$, $P<0.0001$), SDA ($F = 198.06$, $df = 5$, $P<0.0001$), PDA ($F = 29.04$, $df = 5$, $P<0.0001$). Media by isolate interaction was significant ($F = 29.72$, $df = 15$, $P<0.0001$) (Fig. 4b). Isolates MM, ICIPE 30, DLCO 43 and DLCO 41 were germinated at the range of 94.67-100% on all evaluated media. The lowest germination percentage was recorded from isolate DLCO 105 (73% on SDA and 83.33% on MPDA) followed from that of isolate DLCO 87 (85.67%) on SDA. Isolate DLCO 105 and DLCO 87 showed more than 90% germination in all media tested. After twenty four hours of incubation, all isolates, except DLCO 105 (86.67%) on SDA, germinated ≥ 94% on all tested media (Fig. 5).

4.2.2 Radial growth

There were a highly significant differences on radial growth after 12 days between isolate on MPDA ($F = 286.18$, $df = 5$, $P<0.0001$), MEA ($F = 185.87$, $df = 5$, $P<0.0001$), SDA ($F = 57.36$, $df = 5$, $P<0.0001$), PDA ($F = 901.20$, $df = 5$, $P<0.0001$). Media by isolate interaction was significant ($F = 68.73$, $df = 20$, $P<0.0001$) (Fig. 6a). Radial growth ranged between 51.33- 54.67mm for all tested isolates on oat meal agar (OMA). For isolates, MM and ICIPE 30, the recorded highest radial growth was 54 and 55mm while the lowest was 36.67 and 38.33mm on PDA and SDA, respectively. Among Beauveria isolates, the highest radial growth was recorded for isolate DLCO 105 (54.67mm) on OMA. While the lowest was obtained from isolate DLCO 41 (19.33mm) on MPDA. All culture media other than oat meal agar gave a radial growth in the range of 19.33 to 37.67mm for all Beauveria isolates. PDA was found to be the best media for radial growth for isolates MM and ICIPE 30 followed by OMA. The best medium for the radial growth of Beauveria isolates was OMA, followed by PDA and MEA for DLCO 105; MPDA and SDA for DLCO 87; PDA, SDA and MEA for DLCO 43 and MEA and SDA for DLCO 41.

There were a significant differences in growth rate by day after treatment between isolates on media of OMA ($F = 4.82$, df 5, $P<0.0119$), MPDA ($F = 210.99$, $df = 5$, $P<0.0001$), MEA ($F = 137.56$, $df = 5$, $P<0.0001$), SDA ($F = 27.13$, $df = 5$, $P<0.0001$), PDA ($F = 785.24$, $df = 5$, $P<0.0001$). Media by isolate interaction was significant ($F = 60.35$, $df = 20$, $P<0.0001$) (Fig. 6b). The Metarhizium isolates, MM and ICIPE 30 showed a radial growth rate of 3.86 and 4.03mm day $^{-1}$ on OMA and PDA, respectively.

The slowest growth rate 2.44 and 2.56mm day^{-1} was recorded on SDA for MM and ICIPE 30, respectively. As results indicate for Beauveria isolates, a high growth rate was observed on OMA (4.28, 4.06, 3.92 and 3.89mm day^{-1}) by isolate DLCO 105, DLCO 41, DLCO 87 and DLCO 43, respectively. Among the tested isolates, DLCO 41 gave the slowest growth rate 1.17mm day^{-1} on MPDA.

4.2.3 Sporulation

There were a highly significant differences on sporulation between isolate on OMA ($F = 1861.65$, $df = 5$, $P<0.0001$), MPDA ($F = 522.46$, $df = 5$, $P<0.0001$), MEA ($F = 591.56$, $df = 5$, $P<0.0001$), SDA ($F = 1054.27$, $df = 5$, $P<0.0001$), PDA ($F = 2342.20$, $df = 5$, $P<0.0001$). Media by isolate interaction was significant ($F = 1257.24$, $df = 20$, $P<0.0001$) (Fig. 7). Two of the Metarhizium isolates (MM and ICIPE 30) showed high sporulation on OMA and SDA. Isolate MM also showed a high sporulation on PDA. The lowest sporulation (1.93×10^8 conidia ml^{-1}) for isolates MM and ICIPE 30 was recorded on MEA. Among the tested isolates, DLCO 105 showed the lowest sporulation (6.10×10^7 conidia ml^{-1}) on MEA and the highest (1.26×10^9 conidia ml^{-1}) on SDA. Among the tested culture media, MEA yielded the lowest sporulation recorded for all tested isolates. Isolate DLCO 87, DLCO 43 and DLCO 41 showed high sporulation, 5.51×10^8, 4.20×10^8 and 6.85×10^8 conidia ml^{-1}on SDA, PDA and OMA, respectively.

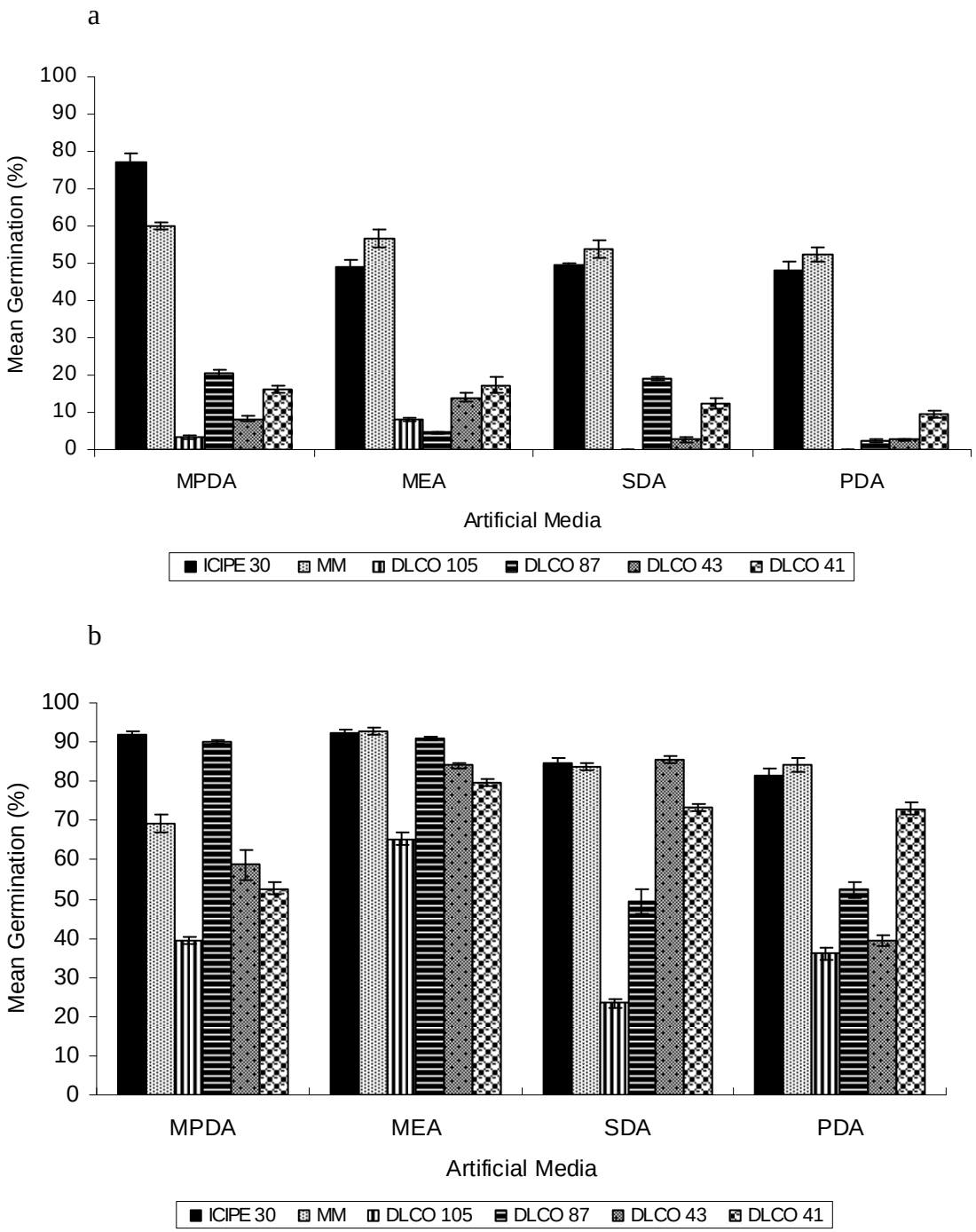


Figure 3. The effect of artificial media on mean germination (%) after (a) 8 hour (b) 12 hour

MPDA=Malt extract peptone dextrose agar, MEA= Malt extract agar, SDA= Sabouraud dextrose agar, PDA= Potato dextrose agar. ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

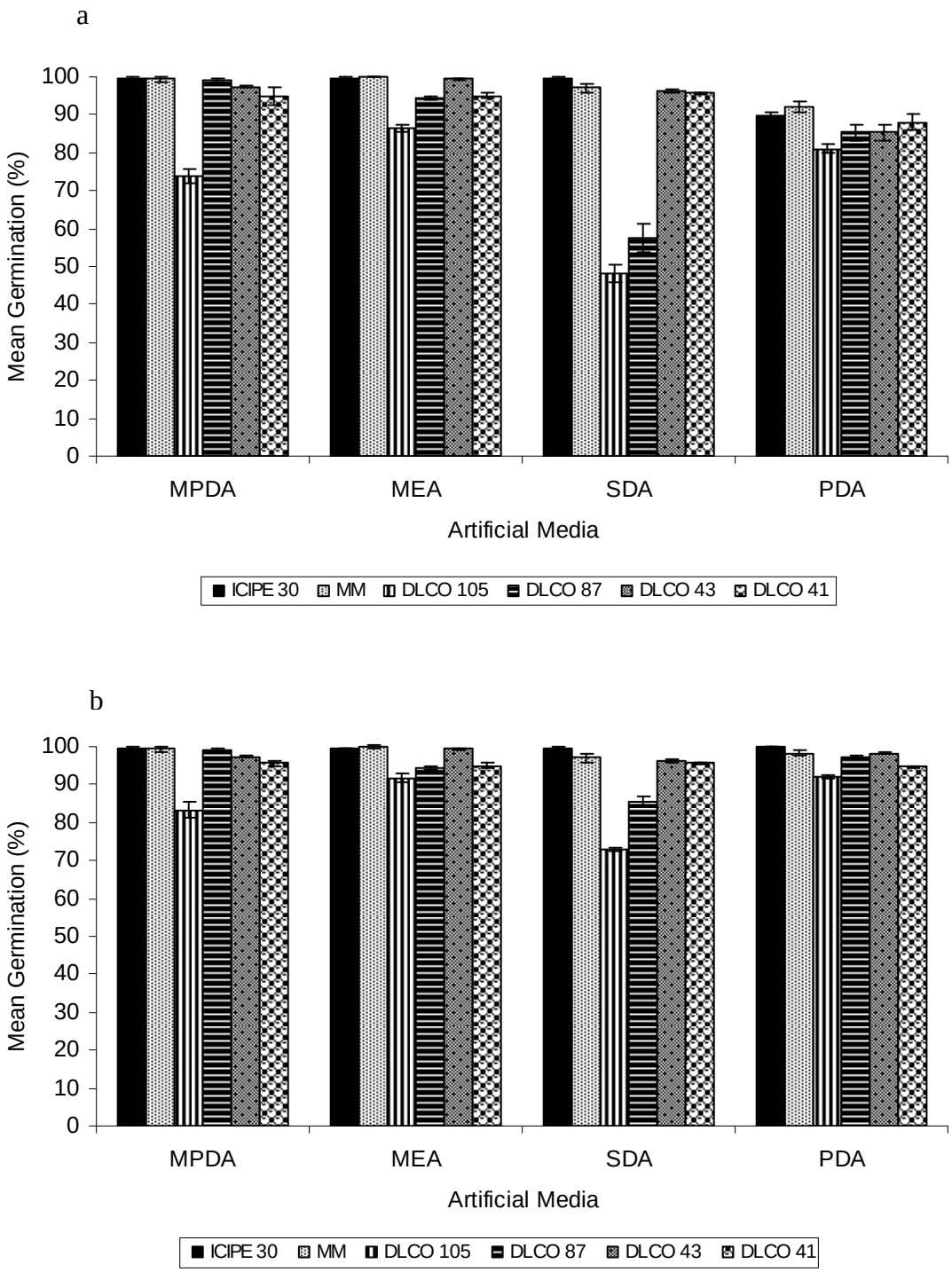


Figure 4. The effect of artificial media on mean germination (%) after (a) 16 hour (b) 20 hour

MPDA=Malt extract peptone dextrose agar, MEA= Malt extract agar, SDA= Sabouraud dextrose agar, PDA= Potato dextrose agar. ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

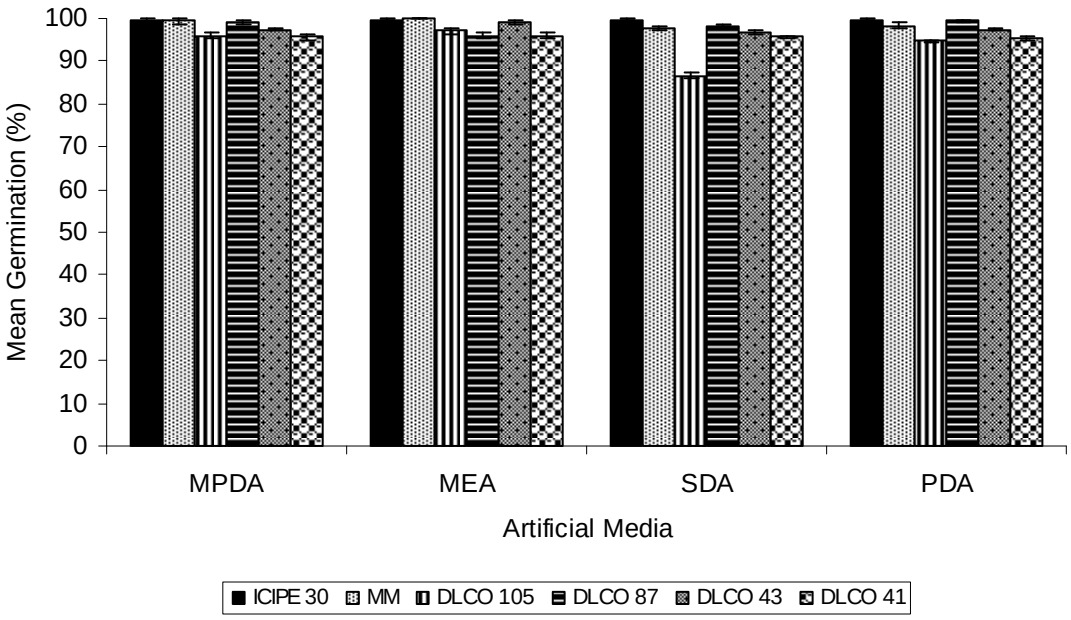


Figure 5. The effect of artificial media on mean germination (%) after 24 hour

MPDA=Malt extract peptone dextrose agar, MEA= Malt extract agar, SDA= Sabouraud dextrose agar, PDA= Potato dextrose agar. ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

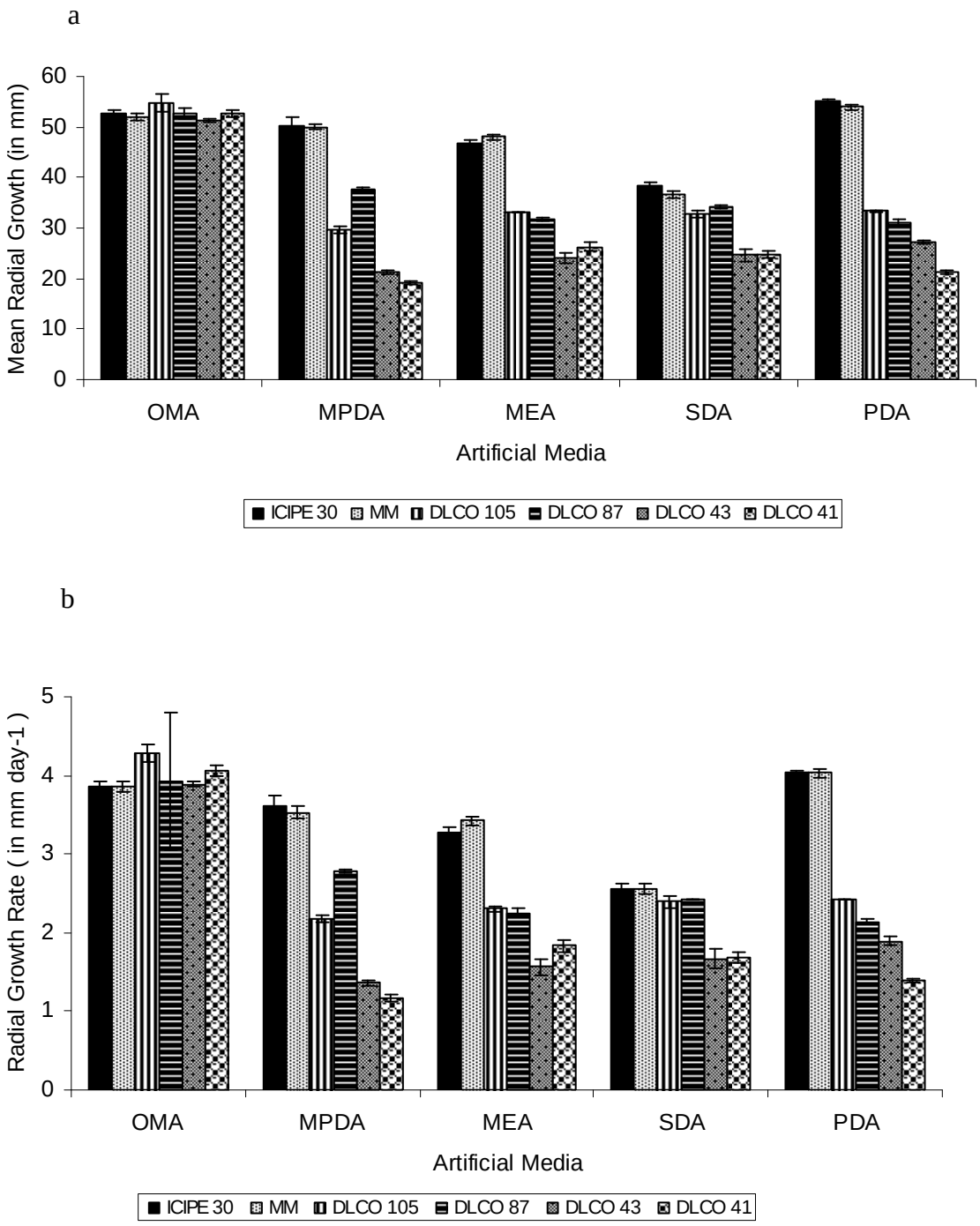


Figure 6. The effect of artificial media on (a) radial growth and (b) growth rate day-1 after 12 days

MPDA=Malt extract peptone dextrose agar, MEA= Malt extract agar, SDA= Sabouraud dextrose agar, PDA= Potato dextrose agar. ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

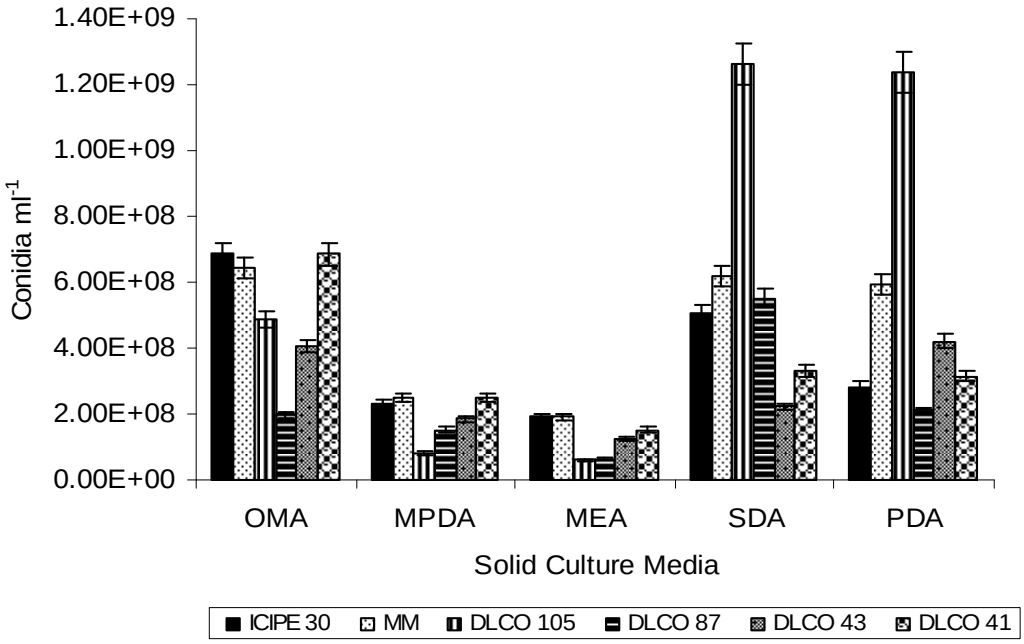


Figure 7. The effect of artificial media on sporulation after 12 days

MPDA=Malt extract peptone dextrose agar, MEA= Malt extract agar, SDA= Sabouraud dextrose agar, PDA= Potato dextrose agar. ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

4.3 Study in pathogenicity

4.3.1 Mean percentage mortality of *Aphis gossypii* treated with isolate ICIPE 30 at different temperature and conidial concentration over time

Percent mortality of *Aphis gossypii* at 1 x 10^8 conidia ml^{-1} ranged between (44.45-86.67%), (28.89-82.22%) and (6.67-68.89%) and for 1 x 10^7 conidia ml^{-1}, percent mortality was ranged between (22.22-60.00), (17.78-55.55%) and (0.00-35.56%) at 30, 25 and 20^0c, respectively (Tables 1 and 2).

Isolate ICIPE 30 showed a significant differences between concentration in cumulative mortality against *Aphis gossypii* at 30^0c on day 3 ($F = 60.14$, $df = 4$, $P<0.0001$), 4 ($F = 89.35$, $df = 4$, $P<0.0001$), 5 ($F = 139.90$, $df = 4$, $P<0.0001$) and 6 ($F = 189.66$, $df = 4$, $P<0.0001$); at 25^0c on day 3 ($F = 20.50$, $df = 4$, $P<0.0001$), 4 ($F = 101.30$, $df = 4$, $P<0.0001$), 5 ($F = 80.57$, $df = 4$, $P<0.0001$) and 6 ($F = 145.04$, $df = 4$, $P<0.0001$); at 20^0c on day 4 ($F = 4.39$, $df = 4$, $P<0.03$), 5 ($F = 17.57$, $df = 4$, $P<0.0002$) and 6 ($F = 20.21$, $df = 4$, $P<0.0001$). There were a significant differences between temperature for 1 x 10^8 conidia ml^{-1} on day 3 ($F = 19.91$, $df = 2$, $P<0.002$), 4 ($F = 17.12$, $df = 2$, $P<0.003$), 5 ($F = 19.50$, $df = 2$, $P<0.002$) and 6 ($F = 6.50$, $df = 2$, $P<0.032$); for 1 x 10^7 conidia ml^{-1} on day 3 ($F = 10.49$, $df = 2$, $P<0.01$), 4 ($F = 41.12$, $df = 2$, $P<0.0003$), 5 ($F = 15.31$, $df = 2$, $P<0.004$) and 6 ($F = 12.87$, $df = 2$, $P<0.007$) (Tables 1 and 2). On days 5 and 6 post inoculation, a significant difference in percent mortality of *Aphis gossypii* was recorded between concentrations of 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at all temperatures. Similarly on day 3 at 20^0c and on day 4 at both 25 and 30^0c a significant difference in percent mortality was recorded between concentrations of 1 x 10^8 and 1 x

10^7 conidia ml^{-1} and the control. A significant difference in mortality was also recorded between 25 and 20^0c and 30 and 20^0c for 1 x 10^8 and 1 x 10^7 conidia ml^{-1} on days 3, 4 and 5 post inoculations. Similarly on day six for 1 x 10^7 conidia ml^{-1} between 25 and 20^0c and 30 and 20^0c and for 1 x 10^8 conidia ml^{-1} between 30 and 20^0c, a significant difference in mortality was also recorded.

Table 1. Mean mortality (%) by day three and four post treatment of *Aphis gossypii* treated with isolate ICIPE 30 at different temperature and concentration.

Concentration (conidia/ml)	Day three (Mean ± SE)			Day four (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	6.67 ± 6.67 (a)B	28.89 ± 2.22 (a)A	44.45 ± 2.22 (a)A	22.22 ± 8.89(a)B	53.33 ± 0.00 (a)A	64.45 ± 2.22 (a)A
1 x 10^7	0.00 ± 0.00 (a)B	17.78 ± 4.44 (ab)A	22.22 ±4.44 (b)A	4.45 ± 2.22 (ab)B	35.55 ± 2.22(b) A	42.22 ± 4.44 (b)A
1 x 10^6	0.00 ± 0.00 (a)B	8.89 ± 2.22 (bc)A	13.33 ± 0.00 (bc)A	2.22 ± 2.22 (ab)B	11.11 ± 4.44 (c)AB	24.45 ± 2.22 (c)A
1 x 10^5	0.00 ± 0.00 (a)B	2.22 ± 2.22 (c)AB	6.67 ± 0.00 (cd)A	2.22 ± 2.22 (ab)B	6.67 ± 0.00 (cd)B	15.55 ± 2.22 (c)A
0	0.00 ± 0.00 (a)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

Table 2. Mean mortality (%) by day five and six post treatment of *Aphis gossypii* treated with isolate ICIPE 30 at different temperature and concentration.

Concentration (conidia/ml)	Day five (Mean ± SE)			Day six (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	48.89 ± 5.87 (a)B	71.11 ± 2.22 (a)A	80.00 ± 0.00 (a)A	68.89 ± 5.87 (a)B	82.22 ± 2.22 (a)AB	86.67 ± 0.00 (a)A
1 x 10^7	20.00 ± 3.85 (b)B	48.89 ± 5.87 (b)A	53.33 ±3.84 (b)A	35.56 ± 5.87 (b)B	55.55 ± 2.22 (b)A	60.00 ± 0.00 (b)A
1 x 10^6	6.67 ± 6.67 (b)B	11.11 ± 4.44 (c)AB	33.33 ± 3.84 (c)A	15.55 ± 9.68 (bc)A	17.78 ± 4.44 (c)A	37.77 ± 4.44 (c)A
1 x 10^5	6.67 ± 3.84 (b)A	6.67 ± 0.00 (c)A	17.78 ± 2.22 (d)A	13.33 ± 3.84 (bc)A	11.11 ± 2.22 (cd)A	17.78 ± 2.22 (d)A
0	0.00 ± 0.00 (b)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (e)A	0.00 ± 0.00 (c)A	2.22 ± 2.22 (d)A	2.22 ± 2.22 (e)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

4.3.2 Mean percentage mortality of *Aphis gossypii* treated with isolate MM at different temperature and conidial concentration over time

Percent mortality of *A. gossypii* treated with 1 x 10^8 conidia ml^{-1} ranged between (26.67-84.44%), (31.11-75.56%) and (6.67-60.00%) and for 1 x 10^7 conidia ml^{-1} it was ranged between (17.68-62.22%), (15.55-48.89%) and (4.45-33.33%) at 30, 25 and 20^0c, respectively (Tables 3 and 4).

Isolate MM showed a significant differences between concentration in cumulative mortality against *Aphis gossypii* at 30^0c on day 3 ($F = 21.31$, $df = 4$, $P<0.0001$), 4 ($F = 43.84$, $df = 4$, $P<0.0001$), 5 ($F = 58.12$, $df = 4$, $P<0.0001$) and 6 ($F = 76.16$, $df = 4$, $P<0.0001$); at 25^0c on day 3 ($F = 86.78$, $df = 4$, $P<0.0001$), 4 ($F = 129.63$, $df = 4$, $P<0.0001$), 5 ($F = 59.34$, $df = 4$, $P<0.0001$) and 6 ($F = 53.96$, $df = 4$, $P<0.0001$); at 20^0c on day 4 ($F = 24.85$, $df = 4$, $P<0.0001$), 5 ($F = 32.26$, $df = 4$, $P<0.0001$) and 6 ($F = 41.36$, $df = 4$, $P<0.0001$). There were a significant differences between temperature for 1 x 10^8 conidia ml^{-1} on day 3 ($F = 103.18$, $df = 2$, $P<0.0001$), 4 ($F = 47.99$, $df = 2$, $P<0.0002$), 5 ($F = 6.64$, $df = 2$, $P<0.03$) and 6 ($F = 6.64$, $df = 2$, $P<0.03$); for 1 x 10^7 conidia ml^{-1} on day 3 ($F = 10.32$, $df = 2$, $P<0.01$), 4 ($F = 14.27$, $df = 2$, $P<0.005$), 5 ($F = 13.47$, $df = 2$, $P<0.006$) and 6 ($F = 9.07$, $df = 2$, $P<0.015$) (Tables 3 and 4). On days 4, 5, and 6 post treatment, a significant difference in mortality was observed between concentrations 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at all temperature. Similarly on day 3 at 25^0c a significant difference in mortality was observed between concentrations 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control (Tables 3 and 4). A significant difference in mortality was recorded between temperatures 20 and 30^0c for 1 x 10^8 and 1 x 10^7 conidia ml^{-1} on

days 3, 4, 5 and 6 post inoculations. Similarly between temperatures 20 and 25^0c for 1 x 10^8 and 1 x 10^7 conidia ml^{-1} a significant difference in mortality was recorded on days 3 and 4 post inoculation, while on day 5 post inoculation a significant difference in mortality was recorded between 20 and 25^0c for 1 x 10^7 conidia ml^{-1}.

Table 3. Mean mortality (%) by day three and four post treatment of *Aphis gossypii* treated with isolate MM at different temperature and concentration.

Concentration (conidia/ml)	Day three (Mean ± SE)			Day four (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	6.67 ± 0.00 (a)B	31.11 ± 2.22 (a)A	26.67 ± 0.00 (a) A	28.89 ± 2.22 (a)B	55.55 ± 2.22 (a)A	55.55 ± 2.22 (a)A
1 x 10^7	4.45 ± 2.22 (a)B	15.55 ± 2.22 (b)A	17.78 ± 2.22 (ab)A	4.45 ± 2.22 (b)B	33.33 ± 3.84 (b)A	31.11 ± 5.87 (b)A
1 x 10^6	4.44 ± 4.44 (a)A	6.67 ± 0.00 (c)A	11.11 ± 2.22 (bc)A	4.44 ± 4.44 (b)A	13.33 ± 0.00 (c)A	17.55 ± 2.22 (bc)A
1 x 10^5	0.00 ± 0.00 (a)A	0.00 ± 0.00 (d)A	6.67 ± 3.84 (cd)A	0.00 ± 0.00 (b)C	6.67 ± 0.00 (cd)B	17.78 ± 2.22 (c)A
0	0.00 ± 0.00 (a)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

Table 4. Mean mortality (%) by day five and six post treatment of *Aphis gossypii* treated with isolate MM at different temperature and concentration.

Concentration (conidia/ml)	Day five (Mean ± SE)			Day six (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	53.33 ± 3.84 (a)B	68.89 ± 5.87 (a)AB	77.78 ± 4.44 (a)A	60.00 ± 3.85 (a)B	75.56 ± 5.87 (a)AB	84.44 ± 4.44 (a)A
1 x 10^7	15.56 ± 4.44 (b)B	42.22 ± 4.44 (b)A	53.33 ±6.66 (b)A	33.33 ± 3.84 (b)B	48.89 ± 5.87 (b)AB	62.22 ± 4.44 (b)A
1 x 10^6	8.89 ± 5.87 (b)A	17.78 ± 2.22 (c)A	20.00 ± 3.85 (c)A	11.11 ± 5.87 (c)A	20.00 ± 0.00 (c)A	31.11 ± 4.44 (c)A
1 x 10^5	6.67 ± 0.00 (b)B	11.11 ± 2.22 (cd)AB	17.78 ± 2.22 (cd)A	11.11 ± 2.22 (c)B	17.78 ± 2.22 (c)AB	24.45 ± 2.22 (c)A
0	0.00 ± 0.00 (b)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (c)A	2.22 ± 2.22 (c)A	2.22 ± 2.22 (d)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

4.3.3 Mean percentage mortality of *Aphis gossypii* treated with isolate DLCO 105 at different temperature and conidial concentration over time

The recorded percent mortality of *Aphis gossypii* subjected to treatment with 1 x 10^8 conidia ml^{-1} ranged between (28.29-82.22%), (6.67-73.33%) and (6.67-62.22%) and for 1 x 10^7 conidia ml^{-1} it was ranged between (15.55-57.78%), (6.67-51.11%) and (0.00-48.89%) at 30, 25 and 20^0c, respectively (Tables 5 and 6) .

Isolate DLCO 105 showed a significant differences between concentration in cumulative mortality against *Aphis gossypii* at 30^0c on day 3 ($F = 24.76$, $df = 4$, $P<0.0001$), 4 ($F = 33.39$, $df = 4$, $P<0.0001$), 5 ($F = 45.91$, $df = 4$, $P<0.0001$) and 6 ($F = 72.72$, $df = 4$, $P<0.0001$); at 25^0c on day 4 ($F = 13.72$, $df = 4$, $P<0.0005$), 5 ($F = 60.18$, $df = 4$, $P<0.0001$) and 6 ($F = 94.58$, $df = 4$, $P<0.0001$); at 20^0c on day 4 ($F = 22.70$, $df = 4$, $P<0.0001$), 5 ($F = 107.88$, $df = 4$, $P<0.0001$) and 6 ($F = 186.59$, $df = 4$, $P<0.0001$). There were a significant differences between temperature for 1 x 10^8 conidia ml^{-1} on day 3 ($F = 14.29$, $df = 2$, $P<0.005$), 4 ($F = 13.08$, $df = 2$, $P<0.007$), 5 ($F = 9.37$, $df = 2$, $P<0.01$) and 6 ($F = 7.62$, $df = 2$, $P<0.02$); for 1 x 10^7 conidia ml^{-1} on day 3 ($F = 9.25$, $df = 2$, $P<0.015$), 5 ($F = 4.90$, $df = 2$, $P<0.05$) (Tables 5 and 6). On days 3, 4, 5 and 6 post treatment, a significant difference in mortality was recorded between concentrations, 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at 30^0c. Similar significant result was also observed between concentrations 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control on day 6 at 20 and 25^0c. Similarly on day 5 at 20^0c a significant difference in mortality was recorded between concentrations 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control. A significant difference in mortality was recorded between temperatures of 20 and 30^0c and 25 and

30^0c for 1 x 10^8 conidia ml^{-1} on days 3, 4, and 5 post treatment. Similarly on day 3 and 5 post treatment, a significant difference in mortality was recorded between temperatures of 20 and 30^0c for 1 x 10^7 conidia ml^{-1}. On day 6 post inoculation, a significant difference was recorded between temperatures of 20 and 30^0c for 1 x 10^8 conidia ml^{-1}.

Table 5. Mean mortality (%) by day three and four post treatment of *Aphis gossypii* treated with isolate DLCO 105 at different temperature and concentration

Concentration (conidia/ml)	Day three (Mean ± SE)			Day four (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	6.67 ± 3.84 (a)B	6.67 ± 0.00 (a)A	28.89 ± 4.44 (a)A	24.44 ± 4.44 (a)B	24.44 ± 2.22 (a)B	51.11 ± 5.87 (a)A
1 x 10^7	0.00 ± 0.00 (a)B	6.67 ± 3.84 (a)AB	15.55 ± 2.22 (b)A	15.55 ± 2.22 (ab)A	22.22 ± 4.44 (a)A	28.89 ± 4.44 (b)A
1 x 10^6	0.00 ± 0.00 (a)B	4.45 ± 2.22 (a)AB	8.89 ± 2.22 (bc)A	6.67 ± 0.00 (bc)A	6.67 ± 3.84 (b)A	20.00 ± 3.85 (b)A
1 x 10^5	0.00 ± 0.00 (a)A	0.00 ± 0.00 (a)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (c)A	4.45 ± 2.22 (b)A	0.00 ± 0.00 (c)A
0	0.00 ± 0.00 (a)A	0.00 ± 0.00 (a)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (c)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

Table 6. Mean mortality (%) by day five and six post treatment of *Aphis gossypii* treated with isolate DLCO 105 at different temperature and concentration.

Concentration (conidia/ml)	Day five (Mean ± SE)			Day six (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	46.67 ± 3.84 (a)B	51.11 ± 2.22 (a)B	71.11 ± 5.87 (a)A	62.22 ± 2.22 (a)B	73.33 ± 3.84 (a)AB	82.22 ± 4.44 (a)A
1 x 10^7	33.33 ± 0.00 (b)B	40.00 ± 3.85 (a)AB	51.11 ± 5.87 (b)A	48.89 ± 2.22 (b)A	51.11 ± 2.22 (b)A	57.78 ± 5.87 (b)A
1 x 10^6	15.55 ± 2.22 (c)B	17.78 ± 2.22 (b)B	37.78 ± 4.44 (b)A	31.11 ± 2.22 (c)B	24.45 ± 2.22 (c)B	42.22 ± 2.22 (b)A
1 x 10^5	0.00 ± 0.00 (d)B	6.67 ± 3.84 (bc)AB	13.33 ± 0.00 (c)A	4.45 ± 2.22 (d)B	13.33 ± 3.84 (cd)AB	17.78 ± 2.22 (c)A
0	0.00 ± 0.00 (d)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (d)A	2.22 ± 2.22 (d)A	2.22 ± 2.22 (c)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

4.3.4 Mean percentage mortality of *Aphis gossypii* treated with isolate DLCO 87 at different temperature and conidial concentration over time

Percent mortality of *Aphis gossypii* treated with 1 x 10^8 conidia ml^{-1} ranged between (4.45-100%), (8.89-88.89%) and (6.67-80.00%) while at 1 x 10^7 conidia ml^{-1} mortality ranged between (17.78-77.78%), (2.22-62.22%) and (0.00-53.33%) at 30, 25 and 20^0c, respectively (Tables 7 - 8).

Isolate DLCO 87 showed a significant differences between concentration in cumulative mortality against *Aphis gossypii* at 30^0c on day 3 ($F = 60.69$, $df = 4$, $P<0.0001$), 4 ($F = 114.67$, $df = 4$, $P<0.0001$), 5 ($F = 288.45$, $df = 4$, $P<0.0001$) and 6 ($F = 279.05$, $df = 4$, $P<0.0001$); at 25^0c on day 3 ($F = 4.50$, $df = 4$, $P<0.025$), 4 ($F = 46.02$, $df = 4$, $P<0.0001$), 5 ($F = 95.43$, $df = 4$, $P<0.0001$) and 6 ($F = 118.07$, $df = 4$, $P<0.0001$); at 20^0c on day 4 ($F = 161.59$, $df = 4$, $P<0.0001$), 5 ($F = 287.47$, $df = 4$, $P<0.0001$) and 6 ($F = 163.46$, $df = 4$, $P<0.0001$). There were a significant differences between temperature for 1 x 10^8 conidia ml^{-1} on day 3 ($F = 28.51$, $df = 2$, $P<0.0009$), 4 ($F = 6.00$, $df = 2$, $P<0.037$), 5 ($F = 6.26$, $df = 2$, $P<0.034$) and 6 ($F = 61.12$, $df = 2$, $P<0.0001$); for 1 x 10^7 conidia ml^{-1} on day 3 ($F = 28.47$, $df = 2$, $P<0.0009$), 4($F = 16.78$, $df = 2$, $P<0.004$), 5 ($F = 25.31$, $df = 2$, $P<0.001$), 6 ($F = 11.62$, $df = 2$, $P<0.009$) (Tables 7 - 8). By days 4, 5 and 6 post inoculation, a significant difference in mortality was observed between concentrations of 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at all temperatures. Similarly on day 3 post treatment, a significant difference in mortality was observed between concentrations of 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at 20 and 30^0c. A significant difference was recorded in mortality between temperatures 20 and 30^0c at 1 x 10^8 conidia ml^{-1} on days 3 and 6 post

inoculation. Similarly on day 6 post inoculation, a significant difference was recorded between temperatures 20 and 25^0c at 1 x 10^8 conidia ml^{-1}. While at 1 x 10^7 conidia ml^{-1} a significant difference was recorded between temperatures 20 and 30^0c and 25 and 30^0c on days 3, 4, and 5 post inoculation. Similarly on day 6 post treatment, a significant difference was recorded between temperatures 20 and 30^0c at 1 x 10^7 conidia ml^{-1}.

Table 7. Mean mortality (%) by day three and four post treatment of *Aphis gossypii* treated with isolate DLCO 87 at different temperature and concentration.

Concentration (conidia/ml)	Day three (Mean ± SE)			Day four (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	6.67 ± 0.00 (a)B	8.89 ± 2.22 (a)B	24.45 ± 2.22 (a)A	44.45 ± 2.22 (a)A	44.44 ± 4.44 (a)A	57.78 ± 2.22 (a)A
1 x 10^7	0.00 ± 0.00 (b)B	2.22 ± 2.22 (ab)B	17.78 ± 2.22 (b)A	22.22 ± 2.22 (b)B	17.78 ± 2.22 (b)B	40.00 ± 3.85 (b)A
1 x 10^6	0.00 ± 0.00 (b)B	2.22 ± 2.22 (ab)AB	6.67 ± 0.00 (c)A	6.67 ± 0.00 (c)C	13.33 ± 0.00 (b)B	22.22 ± 2.22 (c)A
1 x 10^5	0.00 ± 0.00 (b)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (d)A	6.67 ± 0.00 (c)A	11.11 ± 2.22 (bc)A	6.67 ± 0.00 (d)A
0	0.00 ± 0.00 (b)A	0.00 ± 0.00 (a)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (d)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

Table 8. Mean mortality (%) by day five and six post treatment of *Aphis gossypii* treated with isolate DLCO 87 at different temperature and concentration.

Concentration (conidia/ml)	Day five (Mean ± SE)			Day six (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	73.33 ± 0.00 (a)A	73.33 ± 3.84 (a)A	84.45 ± 2.22 (a)A	80.00 ± 0.00 (a)C	88.89 ± 2.22 (a)B	100.00 ± 0.00 (a)A
1 x 10^7	35.55 ± 2.22 (b)B	44.45 ± 2.22 (b)B	57.78 ± 2.22 (b)A	53.33 ± 3.84 (b)B	62.22 ± 4.44 (b)AB	77.78 ± 2.22 (b)A
1 x 10^6	8.89 ± 2.22 (c)C	31.11 ± 2.22 (c)B	48.89 ± 2.22 (b)A	26.67 ± 0.00 (c)C	44.45 ± 2.22 (c)B	64.45 ± 2.22 (c)A
1 x 10^5	15.55 ± 2.22 (c)A	20.00 ± 3.85 (c)A	15.55 ± 2.22 (c)A	20.00 ± 3.85 (c)A	20.00 ± 3.85 (d)A	20.00 ± 3.85 (d)A
0	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	2.22 ± 2.22 (e)A	2.22 ± 2.22 (e)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

4.3.5 Mean percentage mortality of *Aphis gossypii* treated with isolate DLCO 43 at different temperature and conidial concentration over time

Percent mortality of *Aphis gossypii* recorded for 1 x 10^8 conidia ml^{-1} ranged between (13.33-88.89%), (15.55-93.33%) and (6.67-82.22%) and for 1 x 10^7 conidia ml^{-1} death ranged between (13.33-53.33%), (15.55-64.44%) and (4.45-55.55%) at 30, 25 and 20^0c, respectively (Tables 9 - 10).

Isolate DLCO 43 showed a significant differences between concentration in cumulative mortality against *Aphis gossypii* at 30^0c on day 3 ($F = 12.56$, $df = 4$, $P<0.0007$), 4 ($F = 62.27$, $df = 4$, $P<0.0001$), 5 ($F = 45.20$, $df = 4$, $P<0.0001$) and 6 ($F = 109.90$, $df = 4$, $P<0.0001$); at 25^0c on day 3 ($F = 36.70$, $df = 4$, $P<0.0001$), 4 ($F = 86.78$, $df = 4$, $P<0.0001$), 5 ($F = 128.44$, $df = 4$, $P<0.0001$) and 6 ($F = 94.32$, $df = 4$, $P<0.0001$); at 20^0c on day 4 ($F = 46.21$, $df = 4$, $P<0.0001$), 5 ($F = 124.04$, $df = 4$, $P<0.0001$) and 6 ($F = 288.43$, $df = 4$, $P<0.0001$). There were a significant differences between temperature for 1 x 10^7 conidia ml^{-1} on day 4($F = 24.51$, $df = 2$, $P<0.001$), 5 ($F = 6.14$, $df = 2$, $P<0.04$), (Tables 9 - 10). On days 5 and 6 at all temperatures, a significant difference in mortality was observed between spore concentrations, 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control. Similarly on day 4 post inoculation, a significant difference in mortality was observed between spore concentrations, 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at 20 and 30^0c. A significant difference in mortality was recorded between 20 and 25^0c at 1 x 10^7 conidia ml^{-1} on days 4 and 5 post treatment. Similarly on day 4, a significant difference in mortality was also recorded between temperatures 20 and 30^0c at 1 x 10^7 conidia ml^{-1}.

Table 9. Mean mortality (%) by day three and four post treatment of *Aphis gossypii* treated with isolate DLCO 43 at different temperature and concentration.

Concentration (conidia/ml)	Day three (Mean ± SE)			Day four (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	6.67 ± 3.84 (a)A	15.55 ± 2.22 (a)A	13.33 ± 0.00 (a)A	42.22 ± 5.87 (a)A	37.78 ± 2.22 (a)A	51.11 ± 2.22 (a)A
1 x 10^7	4.45 ± 2.22 (a)A	15.55 ± 2.22 (a)A	13.33 ± 3.84 (a)A	20.00 ± 2.22 (b)B	37.78 ± 2.22 (a)A	31.11 ± 2.22 (b)A
1 x 10^6	0.00 ± 0.00 (a)B	0.00 ± 0.00 (b)B	11.11 ± 2.22 (a)A	6.67 ± 0.00 (c)B	20.00 ± 0.00 (b)A	22.22 ± 2.22 (b)A
1 x 10^5	0.00 ± 0.00 (a)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (c)B	8.89 ± 2.22 (c)A	4.45 ± 2.22 (c)AB
0	0.00 ± 0.00 (a)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (c)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

Table 10. Mean mortality (%) by day five and six post treatment of *Aphis gossypii* treated with isolate DLCO 43 at different temperature and concentration.

Concentration (conidia/ml)	Day five (Mean ± SE)			Day six (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	77.78 ± 5.87 (a)A	75.55 ± 2.22 (a)A	71.11 ± 2.22 (a)A	82.22 ± 2.22 (a)A	93.33 ± 3.84 (a)A	88.89 ± 2.22 (a)A
1 x 10^7	44.45 ± 2.22 (b)B	60.00 ± 3.85 (b)A	46.67 ± 3.84 (b)AB	55.55 ± 2.22 (b)A	64.44 ± 4.44 (b)A	53.33 ± 3.84 (b)A
1 x 10^6	13.33 ± 0.00 (c)B	31.11 ± 2.22 (c)A	35.56 ± 5.87 (b)A	24.45 ± 2.22 (c)C	35.55 ± 2.22 (c)B	48.89 ± 2.22 (b)A
1 x 10^5	2.22 ± 2.22 (c)B	24.45 ± 2.22 (c)A	11.11 ± 5.87 (c)AB	11.11 ± 2.22 (d)A	26.67 ± 3.84 (c)A	22.22 ± 4.44 (c)A
0	0.00 ± 0.00 (c)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (e)A	2.22 ± 2.22 (d)A	2.22 ± 2.22 (d)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

4.3.6 Mean percentage mortality of *Aphis gossypii* treated with isolate DLCO 41 at different temperature and conidial concentration over time

Percent mortality of *Aphis gossypii* treated with 1 x 10^8 conidia ml^{-1} ranged between (37.77-95.56%), (11.11-88.89%) and (8.89-71.11%) while in those treated with 1 x 10^7 conidia ml^{-1} mortality ranged between (15.55-55.55%), (13.33-77.78%) and (2.22-48.89%) at 30, 25 and 20^0c, respectively (Tables 11-12).

Isolate DLCO 41 showed a significant differences between concentration in cumulative mortality against *Aphis gossypii* at 30^0c on day 3 ($F = 41.06$, $df = 4$, $P<0.0001$), 4 ($F = 91.59$, $df = 4$, $P<0.0001$), 5 ($F = 164.90$, $df = 4$, $P<0.0001$) and 6 ($F = 216.00$, $df = 4$, $P<0.0001$); at 25^0c on day 3 ($F = 6.08$, $df = 4$, $P<0.01$), 4 ($F = 87.33$, $df = 4$, $P<0.0001$), 5 ($F = 128.30$, $df = 4$, $P<0.0001$) and 6 ($F = 159.58$, $df = 4$, $P<0.0001$); at 20^0c on day 4 ($F = 9.75$, $df = 4$, $P<0.0018$), 5 ($F = 24.88$, $df = 4$, $P<0.0001$) and 6 ($F = 76.64$, $df = 4$, $P<0.0001$). There were a significant differences between temperature for 1 x 10^8 conidia ml^{-1} on day 3 ($F = 26.16$, $df = 2$, $P<0.001$), 4 ($F = 22.07$, $df = 2$, $P<0.002$), 5 ($F = 16.58$, $df = 2$, $P<0.004$) and 6 ($F = 10.78$, $df = 2$, $P<0.01$); for 1 x 10^7 conidia ml^{-1} on day 3 ($F = 6.20$, $df = 2$, $P<0.03$), 4($F = 64.55$, $df = 2$, $P<0.0001$), 5 ($F = 79.84$, $df = 2$, $P<0.0001$), 6 ($F = 46.33$, $df = 2$, $P<0.0002$) (Tables 11-12). On days 3, 4, 5 and 6 post inoculation, a significant difference in mortality was observed based on variations in spore concentration 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at 30^0c. Similarly on days 4, 5 and 6 post treatment, a significant difference in mortality was observed between concentration 1 x 10^8 and 1 x 10^7 conidia ml^{-1} and the control at 20^0c. A significant difference in mortality was recorded between temperatures 20 and 25^0c at 1 x 10^8 and 1 x

10^7 conidia ml^{-1} on days 4, 5 and 6 post inoculation. Similarly on days 3, 4, 5 and 6 post treatment, a significant difference in mortality was also recorded between 20 and 30^0c at 1 x 10^8 conidia ml^{-1}. On day 3 for 1 x 10^8 conidia ml^{-1} and on day 4 for 1 x 10^7 conidia ml^{-1} a significant difference in mortality was recorded between 25 and 30^0c.

Table 11. Mean mortality (%) by day three and four post treatment of *Aphis gossypii* treated with isolate DLCO 41 at different temperature and concentration.

Concentration (conidia/ml)	Day three (Mean ± SE)			Day four (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	8.89 ± 2.22 (a)B	11.11 ± 2.22 (ab)B	37.77 ± 4.44 (a)A	31.11 ± 4.44 (a)B	60.00 ± 3.85 (a)A	75.56 ± 5.87 (a)A
1 x 10^7	2.22 ± 2.22 (a)B	13.33 ± 3.84 (a)AB	15.55 ± 2.22 (b)A	13.33 ± 0.00 (b)C	42.22 ± 2.22 (b)A	31.11 ± 2.22 (b)B
1 x 10^6	4.45 ± 2.22 (a)A	2.22 ± 2.22 (ab)A	8.89 ± 2.22 (bc)A	11.11 ± 5.87 (b)A	13.33 ± 3.84 (c)A	28.89 ± 2.22 (b)A
1 x 10^5	2.22 ± 2.22 (a)A	2.22 ± 2.22 (ab)A	0.00 ± 0.00 (c)A	6.67 ± 3.84 (b)A	13.33 ± 0.00 (c)A	13.33 ± 0.00 (c)A
0	0.00 ± 0.00 (a)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (c)A	0.00 ± 0.00 (b)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (c)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

Table 12. Mean mortality (%) by day five and six post treatment of *Aphis gossypii* treated with isolate DLCO 41 at different temperature and concentration.

Concentration conidia/ml	Day five (Mean ± SE)			Day six (Mean ± SE)		
	20^0c	25^0c	30^0c	20^0c	25^0c	30^0c
1 x 10^8	55.56 ± 5.87 (a)B	84.45 ± 2.22 (a)A	88.89 ± 4.44 (a)A	71.11 ± 4.44 (a)B	88.89 ± 2.22 (a)A	95.56 ± 4.44 (a)A
1 x 10^7	26.67 ± 3.84 (b)C	77.78 ± 2.22 (a)A	48.89 ± 2.22 (b)B	48.89 ± 2.22 (b)B	77.78 ± 2.22 (a)A	55.55 ± 2.22 (b)B
1 x 10^6	11.11 ± 5.87 (bc)B	44.44 ± 5.87 (b)A	37.78 ± 2.22 (b)A	31.11 ± 4.44 (c)B	55.56 ± 4.44 (b)A	46.67 ± 0.00 (b)AB
1 x 10^5	6.67 ± 3.84 (bc)B	24.45 ± 2.22 (c)A	17.78 ± 2.22 (c)AB	17.78 ± 2.22 (c)B	28.89 ± 2.22 (c)A	20.00 ± 0.00 (c)B
0	0.00 ± 0.00 (c)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	0.00 ± 0.00 (d)A	2.22 ± 2.22 (d)A	2.22 ± 2.22 (d)A

Means followed by the same letter/s within a row (upper case) and column (lower case) on the same day are not significantly different (P > 0.05), Tukey's Studentized Range (HSD) test.

4.3.7 Comparison of all fungal isolates on *Aphis gossypii* treated with 1 x 10^7 and 1 x 10^8 conidia ml^{-1} at each temperature over time

There were a significant differences in mortality between isolates for 1 x 10^7 conidia ml^{-1} at 30^0c on day 6 ($F = 5.81$, $df = 5$, $P<0.006$); at 25^0c on day 3 ($F = 3.44$, $df = 5$, $P<0.037$), 4 ($F = 9.09$, $df = 5$, $P<0.0009$), 5 ($F = 13.22$, $df = 5$, $P<0.0002$), 6 ($F = 7.60$, $df = 5$, $P<0.002$); at 20^0c on day 4 ($F = 17.38$, $df = 5$, $P<0.0001$), 5 ($F = 11.58$, $df = 5$, $P<0.0003$), 6 ($F = 6.55$, $df = 5$, $P<0.0037$) (Fig. 8-9). For 1 x 10^7 conidia ml^{-1}, a significant difference was observed for DLCO 87 (77.78%) as compared with the standard isolate ICIPE 30 (60%) on day 6 at 30^0c (Fig. 8a). For 1 x 10^7conidia ml^{-1} at 25^0c, fungal isolate DLCO 41 (77.78% on days 5 and 6) had a significant difference in mortality as compared with the standard isolate ICIPE 30 (48.89% on day 5 and 55.55% on day 6) post inoculation (Fig. 8b). For 1 x 10^7conidia ml^{-1} at 20^0c all Beauveria isolates had a significant difference in mortality as compared with the standard isolate ICIPE 30 on day 4 post inoculation (Fig. 9). For 1 x 10^7conidia ml^{-1} at 20^0c Beauveria isolates DLCO 43 (44.45% on day 5 and 55.55% on day 6) and DLCO 87 (35.55% on day 5 and 53.33% on day 6) had a significant difference in mortality as compared with the standard isolate ICIPE 30 (20% on day 5 and 35.56% on day 6) post inoculation (Fig. 9).

A significant differences in mortality were observed between isolates for 1 x 10^8 conidia ml^{-1} at 30^0c on day 3 ($F = 14.26$, $df = 5$, $P<0.0001$), 4 ($F = 5.96$, $df = 5$, $P<0.005$), 5 ($F = 3.64$, $df = 5$, $P<0.03$), 6 ($F = 4.37$, $df = 5$, $P<0.02$); at 25^0c on day 3 ($F = 26.75$, $df = 5$, $P<0.0001$), 4 ($F = 23.69$, $df = 5$, $P<0.0001$), 5 ($F = 10.53$, $df = 5$, $P<0.0005$), 6 ($F = 4.90$, $df = 5$, $P<0.01$); at 20^0c on day 5 ($F = 7.70$, $df = 5$, $P<0.0019$), 6 ($F = 6.21$, $df = 5$,

P<0.005) (Fig. 10-11). For 1 x 10^8 conidia ml^{-1} at 25 and 30^0c none of the native fungal isolates had significant difference in mortality as compared with the standard isolate ICIPE 30 (Fig. 10a-b) on all days observed. A significant difference was observed among the Beauveria isolates on days 3, 4 and 5 at 30^0c and on days 4, 5 and 6 at 25^0c (Fig. 10a-b). At 20^0c for 1 x 10^8 conidia ml^{-1}, isolates DLCO 87 (73.33%) and DLCO 43 (77.78%) on day 5 had significant difference in percentage mortality as compared with the standard isolate ICIPE 30 (48.89%) (Fig. 11).

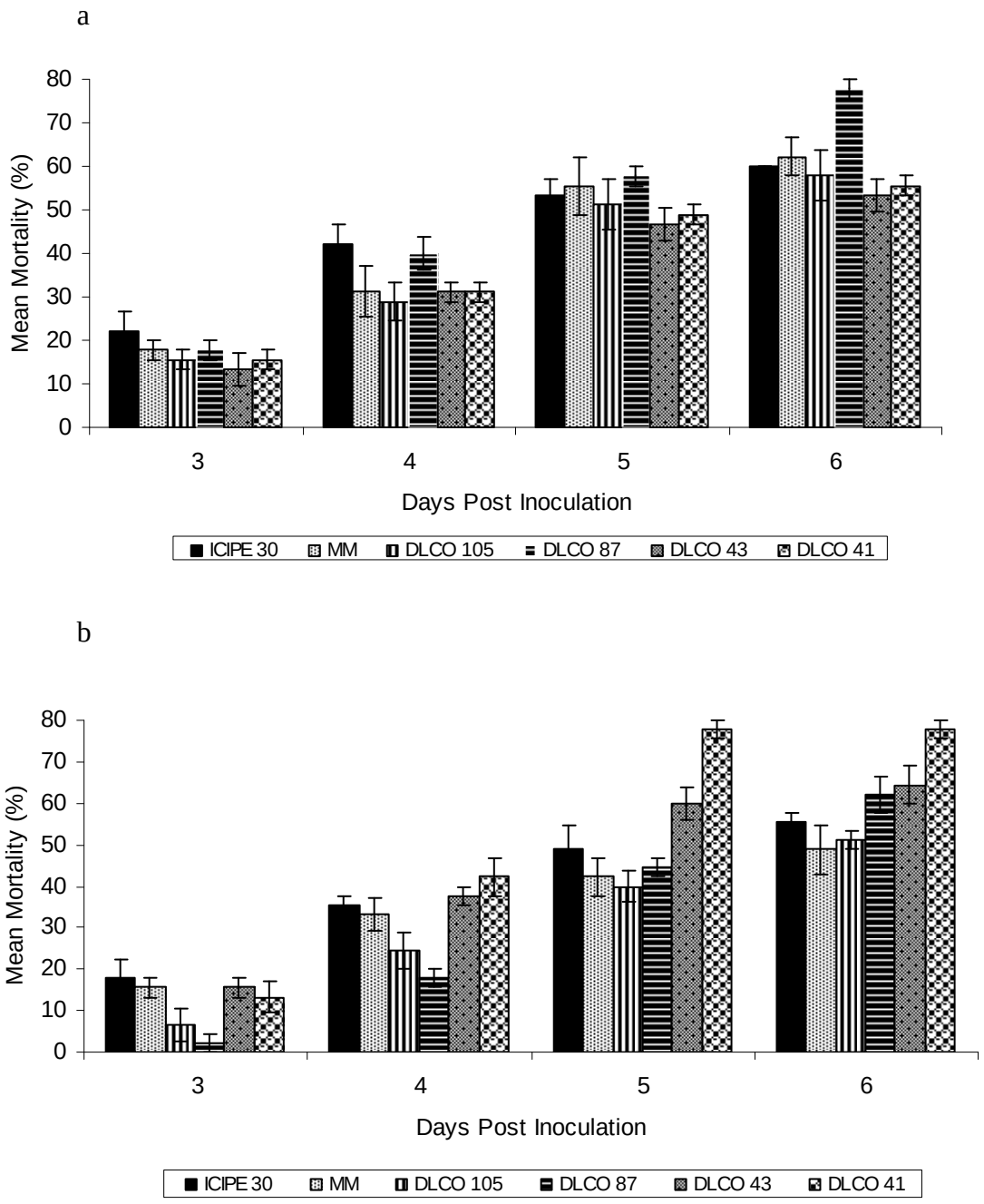


Figure 8. Mean cumulative mortality (%) of *Aphis gossypii* compared at 1 x 10^7 conidia ml^{-1} for all the fungal isolates over time (a) at 30^0c and (b) 25^0c

ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

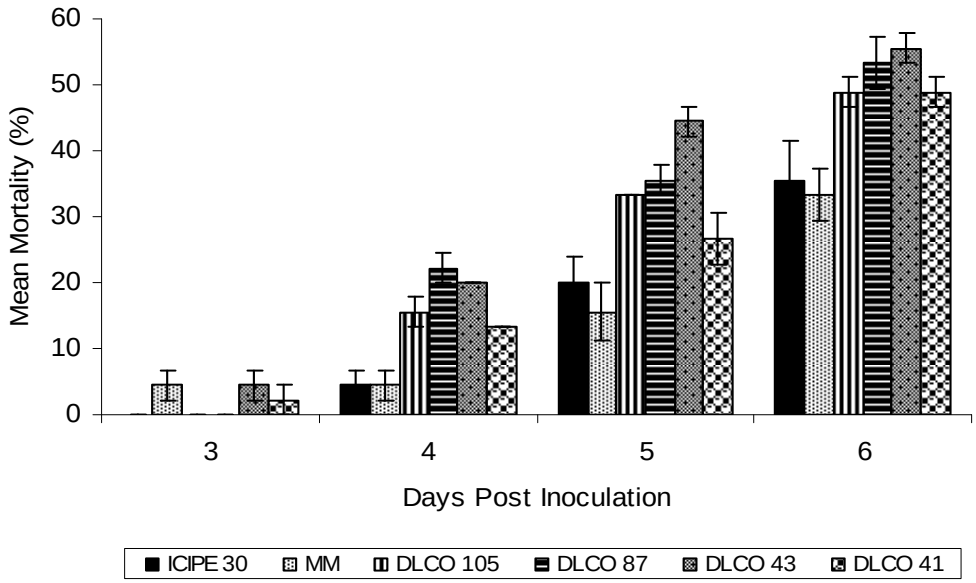
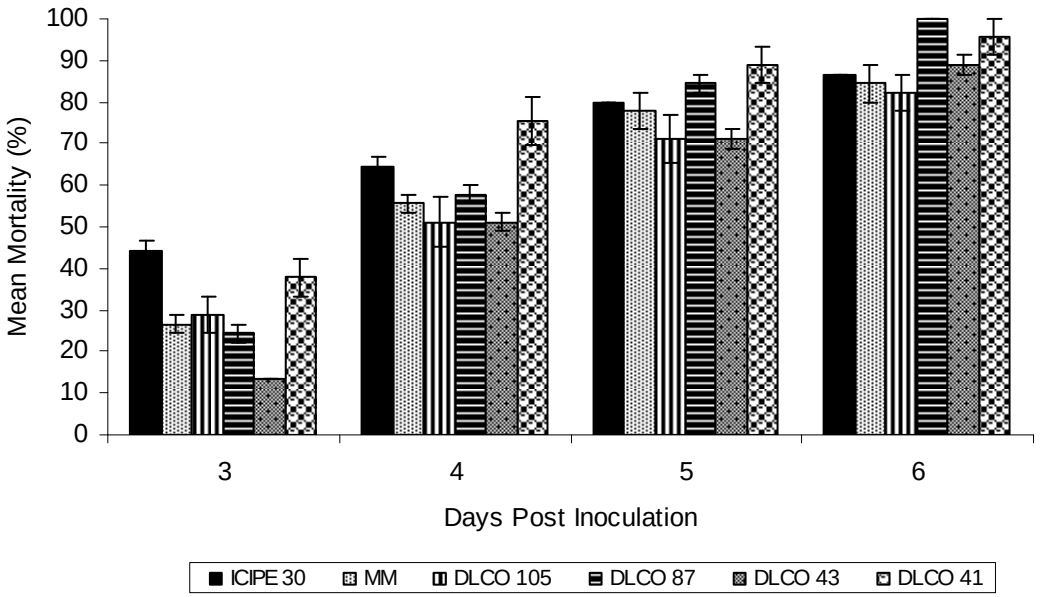


Figure 9. Mean cumulative mortality (%) of *Aphis gossypii* compared at 1 x 10^7 conidia ml^{-1} for all the fungal isolates over time at 20^0c

ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate



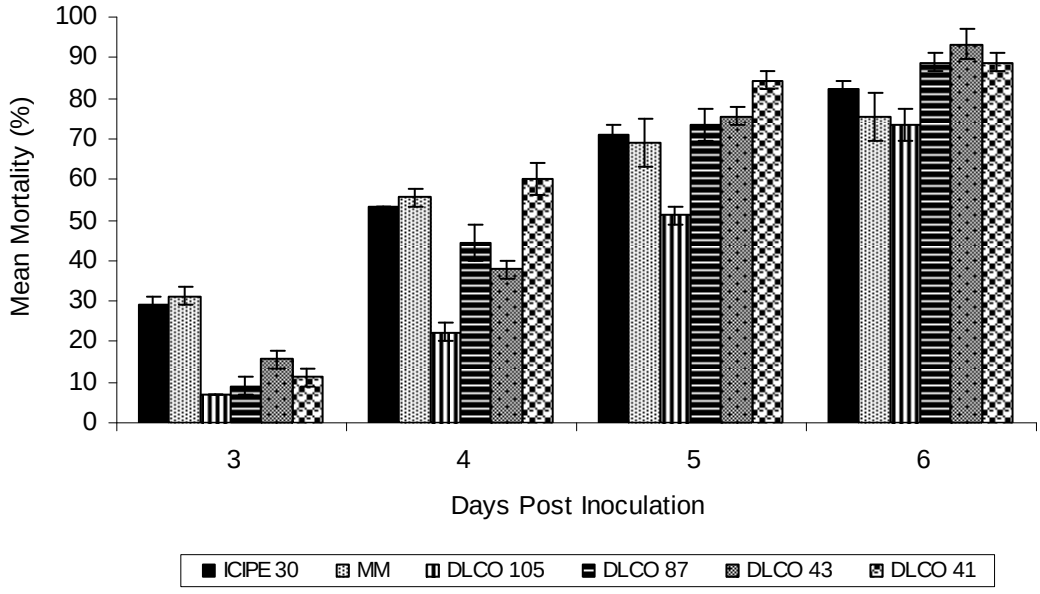


Figure 10. Mean cumulative mortality (%) of *Aphis gossypii* compared at 1 x 10^8 conidia ml^{-1} for all the fungal isolates over time (a) at 30^0c and (b) 25^0c

ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

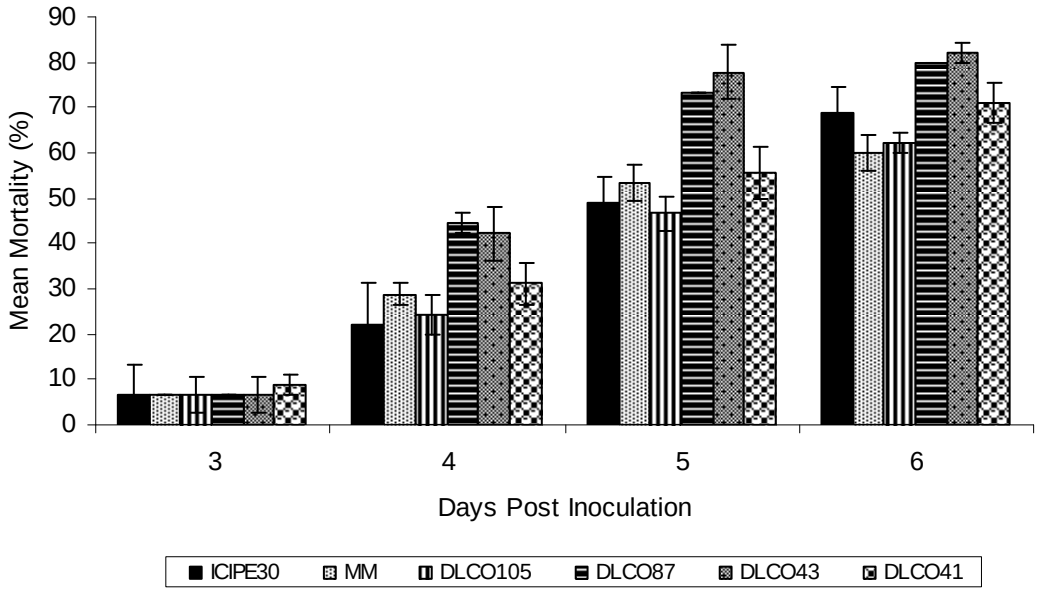


Figure 11. Mean cumulative mortality (%) of *Aphis gossypii* compared at 1 x 10^8 conidia ml^{-1} for all the fungal isolates over time at 20^0c

ICIPE 30= Metarhizium isolate, MM= Metarhizium isolate, DLCO 105 Beauveria isolate, DLCO 87 Beauveria isolate, DLCO 43 Beauveria isolate, DLCO 41 Beauveria isolate

4.3.8 Comparison of corrected mortality (%) of all fungal isolates on *Aphis gossypii* verses conidial concentrations six days post inoculation at each temperature

A significant differences in corrected percentage mortality were observed between isolates after 6 days of inoculation at 30^0c for 1 x 10^8 conidia ml^{-1} (F = 4.46, df = 5, P<0.016), 1 x 10^7 conidia ml^{-1} (F = 6.32, df = 5, P<0.0043), and 1 x 10^6 conidia ml^{-1} (F = 9.71, df = 5, P<0.0007) (Table 13). Isolate DLCO 87 (77.30% and 63.65%) showed a significant difference at both concentration 1 x 10^7 and 1 x 10^6 conidia ml^{-1} as compared with the standard isolate ICIPE 30 (59.05% and 36.19%), respectively.

A significant differences in corrected percentage mortality were observed between isolates after 6 days of inoculation at 25^0c for 1 x 10^8 conidia ml^{-1} (F = 4.82, df = 5, P<0.012), 1 x 10^7 conidia ml^{-1} (F = 6.84, df = 5, P<0.0031), and 1 x 10^6 conidia ml^{-1} (F = 19.24, df = 5, P<0.0001), and 1 x 10^5 conidia ml^{-1} (F = 3.88, df = 5, P<0.025) (Table 14). Isolate DLCO 41 (77.30%, 54.28% and 27.14%) showed a significant difference at concentration 1 x 10^7, 1 x 10^6 and 1 x 10^5 conidia ml^{-1} as compared with the standard isolate ICIPE 30 (54.44%, 16.03 and 9.05%), respectively. Similarly a highly significant difference in percent mortality was recorded at 1 x 10^6 conidia ml^{-1} by isolate DLCO 87 (43.08%) and DLCO 43 (33.90%) as compared with the standard isolate ICIPE 30 (16.03%).

A significant differences in corrected percentage mortality were observed between isolates after 6 days of inoculation at 20^0c for 1 x 10^8 conidia ml^{-1} (F = 6.21, df = 5, P<0.005), 1 x 10^7 conidia ml^{-1} (F = 6.55, df = 5, P<0.004), and 1 x 10^5 conidia ml^{-1} (F =

3.70, df = 5, P<0.03) (Table 15). Both isolates DLCO 43 (55.55%) and DLCO 87 (53.33%) showed a significant difference in percent mortality at 1 x 10^7 conidia ml^{-1} as compared with the standard isolate ICIPE 30 (35.56%).

Table 13. Mean corrected cumulative mortality (%) by day six of *Aphis gossypii* treated with all the fungal isolates verses concentration at 30^0c.

Fungal isolates	Cumulative (%) mortality (corrected) (Mean ± SE)			
	1×10^5	1×10^6	1×10^7	1×10^8
ICIPE 30	15.87 ± 2.08 a	36.19 ± 5.41 bc	59.05 ± 0.95 b	86.35 ± 0.32 ab
MM	22.70 ± 2.07 a	29.37 ± 5.52 c	61.43 ± 4.10 ab	83.97 ± 4.69 b
DLCO 105	15.87 ± 2.08 a	40.79 ± 3.18 bc	56.90 ± 5.30 b	81.90 ± 4.29 b
DLCO 87	18.10 ± 4.29 a	63.65 ± 1.95 a	77.30 ± 2.02 a	100.00 ± 0.00 a
DLCO 43	20.48 ± 3.88 a	47.62 ± 3.05 ab	52.06 ± 4.98 b	88.57 ± 2.39 ab
DLCO 41	18.10 ± 1.90 a	45.40 ± 1.27 bc	54.60 ± 1.27 b	95.56 ± 4.44 ab

Columns associated with the same letter/s are not significantly different ($P > 0.05$), Tukey's Studentized Range (HSD) test.

Table 14. Mean corrected cumulative mortality (%) by day six of *Aphis gossypii* treated with all the fungal isolates verses concentration at 25^0c.

Fungal isolates	Cumulative (%) mortality (corrected) (Mean ± SE)			
	1×10^5	1×10^6	1×10^7	1×10^8
ICIPE 30	9.05 ± 2.14 b	16.03 ± 2.70 d	54.44 ± 2.93 b	81.90 ± 1.90 ab
MM	15.712 ± 4.28 ab	18.10 ± 1.90 cd	47.76 ± 7.02 b	74.76 ± 6.50 b
DLCO 105	11.11 ± 5.87 ab	22.70 ± 2.02 cd	49.84 ± 3.49 b	72.70 ± 3.90 b
DLCO 87	18.25 ± 2.49 ab	43.08 ± 3.65 ab	61.43 ± 4.10 ab	88.57 ± 2.39 ab
DLCO 43	25.08 ± 2.59 ab	33.90 ± 3.31 bc	63.81 ± 3.81 ab	93.33 ± 3.84 a
DLCO 41	27.14 ± 3.44 a	54.28 ± 5.71 a	77.30 ± 2.02 a	88.73 ± 2.06 ab

Columns associated with the same letter/s are not significantly different ($P > 0.05$), Tukey's Studentized Range (HSD) test.

Table 15. Mean corrected cumulative mortality (%) by day six of *Aphis gossypii* treated with all the fungal isolates verses concentration at 20^0c.

Fungal isolates	Cumulative (%) mortality (corrected) (Mean ± SE)			
	1×10^5	1×10^6	1×10^7	1×10^8
ICIPE 30	13.33 ± 3.84 ab	15.15 ± 9.68 a	35.56 ± 5.87 b	68.89 ± 5.87 ab
MM	11.11 ± 2.22 ab	11.11 ± 5.87 a	33.33 ± 3.84 b	60.00 ± 3.85 b
DLCO 105	4.45 ± 2.22 b	31.11 ± 2.22 a	48.89 ± 2.22 ab	62.22 ± 2.22 b
DLCO 87	20.00 ± 3.85 a	26.67 ± 0.00 a	53.33 ± 3.84 a	80.00 ± 0.00 a
DLCO 43	11.11 ± 2.22 ab	24.45 ± 2.22 a	55.55 ± 2.22 a	82.22 ± 2.22 a
DLCO 41	17.78 ± 2.22 ab	31.11 ± 4.44 a	48.89 ± 2.22 ab	71.11 ± 4.44 ab

Columns associated with the same letter/s are not significantly different ($P > 0.05$), Tukey's Studentized Range (HSD) test.

4.3.9 Comparison of LT_{50} and LC_{50} for all the fungal isolates on *Aphis gossypii* at each temperature

For the lower concentration, 1 x 10^5 and 1 x 10^6 conidia ml^{-1} the median lethal time was more than 5 days and went up to 18.95 days. At 1 x 10^8 conidia ml^{-1} less than 4 days of median lethal time was observed from isolate ICIPE 30 (3.82 day), MM (3.89 day) and DLCO 41 (3.95 day) at 25^0c and between all tested isolate except isolate DLCO 41 at 30^0c (Tables 16-17). At 20^0c with concentration 1 x 10^7 and 1 x 10^8 conidia ml^{-1} the lower median lethal time observed was 5.49 and 4.31 days for isolate DLCO 43, respectively (Tables 18).

The resulting LC50 value of each isolate six days post inoculation at all temperature ranges is shown with acceptable Chi square (X^2) values ($P<0.05$) (Tables 19-21). At 30^0c among the tested fungal isolates DLCO 87 had the lowest LC_{50} values (6.78 x 10^5 conidia ml^{-1}). Isolates DLCO 105, ICIPE 30 and MM showed less virulence to *Aphis gossypii* as compared to isolate DLCO 87 at 30^0c (Table 19). At 25^0c, among the tested fungal isolates DLCO 41 had the lowest LC_{50} value (6.89 x 10^5 conidia ml^{-1}). Isolates MM, DLCO 105 and ICIPE 30 showed less virulence to *A. gossypii* as compared to isolate DLCO 41 (Table 20). At 20^0c, the Beauveria isolate DLCO 87 and DLCO 43 showed the lowest LC_{50} value among the other fungal isolates. Three of the fungal isolates MM, ICIPE 30 and DLCO 105 had shown less virulence to *A. gossypii* as compared with isolates DLCO 87 and DLCO 43 (Table 21).

Table 16. LT_{50} in days of six fungal isolates on *Aphis gossypii* verses concentration (conidia ml^{-1}) at 30^0c.

Fungal Isolate	Median lethal time (LT_{50}) ± SE			
	1×10^5	1×10^6	1×10^7	1×10^8
ICIPE 30	10.95 ± 0.79	6.68 ± 0.71	4.81 ± 0.55	3.22 ± 0.49
MM	9.20 ± 0.81	8.37 ± 0.76	4.97 ± 0.74	3.79 ± 0.65
DLCO 105	7.63 ± 2.47	6.21 ± 0.83	5.24 ± 0.60	3.82 ± 0.55
DLCO 87	8.33 ± 1.48	5.17 ± 0.97	4.49 ± 0.79	3.58 ± 0.80
DLCO 43	7.91 ± 1.80	5.97 ± 0.80	5.37 ± 0.75	4.01 ± 0.72
DLCO 41	8.66 ± 1.15	5.88 ± 0.79	5.24 ± 0.73	3.28 ± .74

Table 17. LT_{50} in days of six fungal isolates on *Aphis gossypii* verses concentration (conidia ml^{-1}) at 25^0c.

Fungal Isolate	Median lethal time (LT_{50}) ± SE			
	1×10^5	1×10^6	1×10^7	1×10^8
ICIPE 30	18.49 ± 1.13	16.39 ± 0.66	5.25 ± 0.54	3.82 ± 0.57
MM	11.06 ± 1.51	10.32 ± 0.82	5.67 ± 0.64	3.89 ± 0.53
DLCO 105	10.23 ± 1.77	8.74 ± 1.06	5.69 ± 0.89	4.96 ± 1.03
DLCO 87	8.37 ± 1.22	6.17 ± 1.15	5.34 ± 1.17	4.24 ± 1.03
DLCO 43	7.24 ± 1.34	6.50 ± 1.08	4.73 ± 0.76	4.15 ± 1.01
DLCO 41	7.44 ± 1.06	5.53 ± 1.19	4.28 ± 0.87	3.95 ± 0.99

Table 18. LT_{50} in days of six fungal isolates on *Aphis gossypii* verses concentration (conidia ml^{-1}) at 20^0c.

Fungal Isolate	Median lethal time (LT_{50}) ± SE			
	1×10^5	1×10^6	1×10^7	1×10^8
ICIPE 30	9.27 ± 2.20	8.68 ± 2.26	6.58 ± 1.78	5.08 ± 1.01
MM	18.59 ± 3.13	16.88 ± 1.01	7.61 ± 1.19	5.11 ± 0.90
DLCO 105	10.52 ± 1.23	7.06 ± 1.62	5.87 ± 1.28	5.23 ± 0.94
DLCO 87	8.33 ± 1.48	7.69 ± 1.69	5.66 ± 1.17	4.36 ± 0.97
DLCO 43	8.61 ± 2.00	7.80 ± 1.58	5.49 ± 1.01	4.31 ± 1.02
DLCO 41	11.05 ± 1.23	8.33 ± 1.04	6.10 ± 1.20	4.82 ± 0.91

Table 19. Probit analysis of mortality data of six fungal isolates at 30^0c.

Fungal Isolate	50% Lethal Concentration (LC_{50})				
	Upper	LC_{50}	Lower	X^{2a}	Relative Potency[b]
ICIPE 30	6.11×10^6	2.93×10^6	1.41×10^6	0.4885	100.00
MM	6.51×10^6	2.89×10^6	1.28×10^6	2.1941	101.38
DLCO 105	7.16×10^6	3.16×10^6	1.40×10^6	0.6169	92.72
DLCO 87	1.05×10^6	6.78×10^5	3.71×10^5	4.8828	432.15
DLCO 43	4.65×10^6	2.08×10^6	8.74×10^5	4.5262	140.87
DLCO 41	3.10×10^6	1.90×10^6	2.85×10^6	6.6316	154.21

Analysis was based on data taken six days after inoculation

[a] $P \leq 0.05$, df =2

[b] Relative potency of tested isolates was calculated using the following formula (Feng and Johnson, 1990):

$$\text{Activity of tested isolate} = \frac{LC_{50} \text{ of standard ICIPE 30 (conidia ml}^{-1}\text{)}}{LC_{50} \text{ of tested isolate}} \times 100$$

Table 20. Probit analysis of mortality data of six fungal isolates at 25^0c.

Fungal Isolate	50% Lethal Concentration (LC_{50})				
	Upper	LC_{50}	Lower	X^{2a}	Relative Potency[b]
ICIPE 30	1.49×10^7	7.42×10^6	3.95×10^6	2.2554	100.00
MM	2.66×10^7	9.83×10^6	4.44×10^6	3.0029	75.48
DLCO 105	2.51×10^7	9.73×10^6	4.51×10^6	0.3756	76.26
DLCO 87	4.17×10^6	2.02×10^6	9.28×10^5	0.8790	367.33
DLCO 43	3.52×10^6	1.74×10^6	8.16×10^5	4.0413	426.44
DLCO 41	1.50×10^6	6.89×10^5	2.48×10^5	0.3268	1076.92

Analysis was based on data taken six days after inoculation

[a] $P \leq 0.05$, df =2

[b] Relative potency of tested isolates was calculated using the following formula (Feng and Johnson, 1990):

$$\text{Activity of tested isolate} = \frac{LC_{50} \text{ of standard ICIPE 30 (conidia ml}^{-1}\text{)}}{LC_{50} \text{ of tested isolate}} \times 100$$

Table 21. Probit analysis of mortality data of six fungal isolates at 20^0c.

Fungal Isolate	50% Lethal Concentration (LC_{50})				
	Upper	LC_{50}	Lower	X^{2a}	Relative Potency[b]
ICIPE 30	8.97×10^7	2.53×10^7	1.07×10^7	3.3077	100.00
MM	2.49×10^8	5.05×10^7	1.92×10^7	2.7845	50.10
DLCO 105	5.02×10^7	1.68×10^7	7.48×10^6	3.6258	150.60
DLCO 87	1.39×10^7	5.68×10^6	2.57×10^6	1.9576	445.42
DLCO 43	1.25×10^7	5.75×10^6	2.86×10^6	1.5489	440.00
DLCO 41	2.94×10^7	9.07×10^6	3.65×10^6	0.1614	278.94

Analysis was based on data taken six days after inoculation

[a] $P \leq 0.05$, df =2

[b] Relative potency of tested isolates was calculated using the following formula (Feng and Johnson, 1990):

$$\text{Activity of tested isolate} = \frac{LC_{50} \text{ of standard ICIPE 30 (conidia ml}^{-1}\text{)}}{LC_{50} \text{ of tested isolate}} \times 100$$

5. DISCUSSION

The results of the present study showed that temperature-germination, temperature-growth and temperature-sporulation responses varied considerably between isolates. Optimal temperatures for germination percentage of both the Metarhizium and Beauveria isolates were generally between 20 and 30^0c with only MM and ICIPE 30 exhibiting a germination percentage of 58.33 and 89.67% at temperature of 35^0c, respectively. None of the Beauveria isolates germinated at 35^0c. According to Goral and Lappa (1972) the thermal death point of conidia has been determined as 35^0c. Liu *et al*. (2003) reported a result in line with the present study that among four *Beauveria bassiana* and one *Metarhizium anisopliae* isolates tested, only the *M. anisopliae* isolate had significant spore germination at 35^0c. This is in accord with that by Ferron (1978) in which he explained that for most Metarhizium strains, the optimal temperatures are thought to fall between 23^0c and 25^0c. Similarly, Hywel-Jones and Gillespie (1990) explained that temperatures above or below the optimum range prolong the lag phase and decrease the germination rate. In general, *B. bassiana* can grow at a wide temperature range from 8 to 35^0c, but there are differences based on isolate type. Fargues *et al*. (1997) tested the maximum thermal threshold of 65 isolates of *B. bassiana*. The maximum thermal threshold occurred at temperatures of >35-37^0c for 50 isolates; >32-35^0c for 12; and 30-32^0c for one isolate. In the present study, over 90% of conidia of all isolates germinated after 24 hour at 25 and 30^0c.

According to Shimazu (2004), at 25 to 30^0c, the conidial germination rate rose to almost 100% within 20 hour. At 35^0c, germination was only 4.6% even more than 4 day after

inoculation although most conidia inflated within 24 hour. No germination was observed at 36^0c although most conidia inflated within 24 hour. A study conducted by Liu *et al*. (2003) showed spore viability ranging from 91.4 to 98.6% after incubation for 24 hour at 20^0c for four *Beauveria bassiana* and one *Metarhizium anisopliae* isolates tested, while in the present study, spore germination ranged from 79.00 to 91.33% for the six isolates examined for 24 hour at 20^0c.

Fargues *et al*. (1997) showed that temperature affects the germination rate of *Metarhizium flavoviride* and conidia of *Metarhizium anisopliae* and *Beauveria bassiana* isolates (Hywel-Jones and Gillespie, 1990, Adane kassa, 2003; Tadele and Pringle, 2003). In the present study, the germination capacity of tested isolates were retarded at lower temperature (15^0c) than at 20-30^0c. In line with the present study, Yeo *et al*. (2003) showed that a faster germination rate of conidia of *B. bassiana*, *M. anisopliae*, *Paecilomyces fumosoroseus*, *Verticillium lecanii* and *M. anisopliae* were found better at 25 and 20^0c than at 10 and 15^0c. Similarly, Gillespie and Claydon (1989) reported that *Metarhizium anisopliae* strains vary in their optimum temperatures for spore germination and growth from 20 to 30^0c.

Temperature has been extensively proved to affect mycelium development (Thomas and Blanford, 2003). Fargues *et al*. (1997) and Ouedraogo *et al.* (1997) found different temperature dependent *invitro* growth patterns in isolates of several entomopathogenic fungal species. In the present study, the optimal temperature for radial growth of Metarhizium isolates was 25-30^0c with a maximal radial growth recorded from isolate

ICIPE 30 at 30^0c while at 35^0c despite a high germination percentage radial growth was very much retarded (not more than 2mm in diameter) after 12 days. The best temperature for radial growth of isolate DLCO 105, DLCO 87 and DLCO 43 was found to be at 25^0c, and at 30^0c for DLCO 41. There was an increasing trend in radial growth of the Metarhizium isolates with increase in temperature level from 15 - 30^0c. Different previous studies showed that fungi are highly dependent on ambient microclimate where their growth ranges with optimal between 20 - 30^0c (McCoy *et al*., 1988). Goral and Lappa (1972) reported that the optimal temperature for growth is in general in the range of 25 - 30^0c, minimum 10^0c, and maximum 32^0c apparently depending on the geographic origin of the respective isolate.

The optimal temperature found in this study are similar to previously published results (Hywel-Jones and Gillespie, 1990; Ferron *et al*., 1991; Welling *et al*., 1994; Ouedraogo *et al*., 1997; Fargues *et al*., 1997; Hallsworth and Magan, 1999). Shimazu (2004) investigated mycelial growth and germination rates of *Beauveria bassiana* at various temperatures. Similar to present findings, he reported that mycelial growth was maximal at 30^0c.

The present study showed that the radial growth rate of all tested isolates was increased at 25^0c as compared with 20^0c, but Yeo *et al*. (2003) noted that a decrease in growth rate at 25^0c compared with 20^0c was observed for isolates of *Verticillium lecanii* and *B. bassiana*. The rapidity of mycealial development and evaluation of infection depend on temperature (Kalvish, 1974). Since temperature-growth response varied considerably

between isolates, strain selection according to thermal tolerance may be warranted when choosing a strain for development as a microbial control agent. Our isolates of the present study are tropical in their origins and had shown better growth at higher temperatures of not more than 35^0c. Brooks *et al*. (2004) studied the growth of different fungi of US, Danish, French and Brazilian isolates and observed that none of the isolates grew at 40^0c. The Danish, French and Brazilian isolates grew almost as well at 32^0c and 35^0c as at 28^0c and 30^0c. While, the US isolate declined markedly with temperature. The French and Brazilian isolates showed some growth at 37.5^0c but the Danish and US isolates did not.

Hallsworth and Magan (1999) tested the effect of different temperatures on the growth of *M. anisopliae* and *B. bassiana* and obtained a similar result to the present study in that the near optimal temperature for growth rate for all isolate was at 25^0c, 25^0c was optimal for *B. bassiana* and 30^0c and 35^0c for *M. anisopliae*. However, in the present study at 35^0c no satisfactory growth was obtained for Metarhizium isolates and no growth was recorded for the Beauveria isolates.

The results of the present study showed that after 8 hour, MPDA could favour germination better than all other media for ICIPE 30. A higher germination velocity in some media, at the earlier germination time may be an indication that the composition of a culture medium can influence the germination velocity of conidium. Furthermore, owing to their rich composition probably these media favour the growth of germination. However, the influence of culture media for speed of germination was not reflecting equally between Metarhizium and Beauveria isolates in this study. Dillon and Charnley

(1989) showed that germination of *Metarhizium anisopliae* is initiated by water but progress to the first overt stage of germination (swelling) is dependent on an exogenous nutrient. Moore-Landecker (1972) reported that under laboratory conditions, spore germination may be triggered by the addition of a diverse range of chemical agents including inorganic ions, carbohydrates, amino acids, lipids and vitamins.

The present result showed that the difference between media was more evident for the Beauveria than Metarhizium isolates. A markedly lower germination was observed for DLCO 105 isolate than for others. The highest germination of Beauveria was provided by MPDA, SDA, MEA and PDA differently for different isolates at different time of germination. In a previous study, Francisco *et al.* (2006) reported that the highest germination of *Beauveria bassiana* was provided by PDA, PDAY, SDAY and CM. Smith and Grula (1981) reported that *B. bassiana* requires utilizable carbon source to promote germination and a nitrogen source is needed for continued hyphal growth.

Nutrient rich media used in the present study contain various carbon and nitrogen sources, a factor that might have been decisive in the observation of higher germination percentage. In the current investigation, time for more than 50% germination was recorded for the Metarhizium isolates MM and ICIPE 30 8 hour post inoculation. According to Heale *et al*. (1989) a study conducted on 15 isolates of *M. anisopliae invitro* showed that time for 50% germination ranged from 7-25 to 13 hour on Sabouraud's Dextrose Agar (SDA). Liu *et al.* (2003) also reported that the time required for 50% germination by *Beauveria bassiana* and *Metarhizium anisopliae* isolates on quarter-

strength Sabouraud Dextrose Agar plus 0.25% (w/v) yeast extract was between 14.8 to 18.0 hour at 28^0c. However, the result of the present study showed that when time was extended to 24 hour, no influence of culture medium was observed on the viability of conidia. Germination of conidia was found to be somewhat similar between all isolates after a 24 hour germination time.

As far as mycelial growth of Metarhizium isolates is concerned, the highest value was recorded from PDA, OMA, MPDA whereas culture media of OMA, SDA and PDA showed the highest sporulation. Such variation on mycelial growth and sporulation was also observed between Beauveria isolates. The highest mycelial growth and sporulation was provided by OMA for all isolates and SDA for DLCO 105 and DLCO 87. According to Seneshaw *et al*. (2003) the culture media used for several Ethiopian fungal isolates showed an influence both in colony growth and sporulation. They reported that SDA appeared to favor growth and sporulation for Metarhizium isolate EE. In a previous study, Skrobek (2001) reported a result that culture media did not affect sporulation or viability of spores of *Paecilomyces fumosoroseus* whereas influence of culture medium were observed between the strains of *M. anisopliae* var. *anisopliae*. However, concerning mycelial growth, differences occurred between the strains of *M. anisopliae* var. *anisopliae* and of *P. fumosoroseus*. Liu *et al*. (2003) reported that spore production on quarter-strength Sabouraud Dextrose Agar plus 0.25% (w/v) yeast extract after 10 days incubation at 20^0c ranged from 1.6×10^6 to 15.5×10^6 conidia cm^{-2} and one *B. bassiana* isolate produced significantly more conidia than the others.

For an effective and economic production process of entomopathogen for biological control, rapid growth and a high sporulation rate are as essential as the stable production of viable, infectious conidia (Jenkins *et al*., 1998; Moore and Prior, 1993). Culture conditions can greatly influence the virulence, longevity and ecological fitness of the resultant propagules. Therefore, they can be manipulated to increase mycoinseticide efficiency. Lee *et al*. (1996), Im *et al*. (1988) and Vilas- Boas *et al*. (1996) conducted an experiment on the growth and sporulation of a variety of entomopathagens on different solid culture media. Here in, media that enhanced mycelial growth did not necessarily increase the sporulation rate which was in line with the present study that DLCO 105 recorded 54.67mm radial growth on OMA resulted 1.99 x 10^8 conidia ml^{-1}, while the same isolate recorded 32.67mm radial growth on SDA resulted 1.56 x 10^9 conidia ml^{-1}. Culture conditions can also influence thermal tolerance. McClatchie *et al*. (1994) reported reduced the thermal tolerance of conidia of *M. flavoviride* by increasing the sucrose content of the growth medium.

In the present study, the effect of different concentrations of fungal isolates and temperature demonstrated the susceptibility of *Aphis gossypii*. The results prevailed differences in susceptibility of *A. gossypii* between temperatures and concentrations. The maximum mortality was obtained between 25 and 30^0c at 1 x 10^8 conidia ml^{-1} with all the fungal isolates. However at 20^0c, a mortality percentage of 80% and above was obtained by two of the Beauveria isolates.

The virulence of Metarhizium isolates on adult *A. gossypii* in the present study was influenced by lower temperature more than the Beauveria isolates. In a previous work, it was reported that changes in environmental temperature greatly influence both the speed of kill and over all mortality caused by mitosporic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae* var *acridum* (Blanford and Thomas, 1999; Arthurs and Thomas, 2000 and Blanford and Thomas, 2000). According to Yeo *et al*. (2003), a significant interaction between aphid species and temperature indicated that the pathogenic nature of an isolate was dependent not only on the target aphid species but also the temperature conditions of the bioassay. They also reported that mortality occurred more quickly and reached a maximum earlier at temperatures of 18 and 23^0c as compared with 10^0c. Generally, aphid mortalities observed during one week were entirely dependent on concentration (Yeo *et al*., 2003).

The results of the present study showed that the Beauveria isolates caused 77.30 and 55.55% mortality between 25 and 20^0c at 1 x 10^7 conidia ml^{-1}, respectively. Similarly, a study conducted by Skinner and Parker (2003) on the sucking insect pest, tarnished plant bug, *Lygus lineolaris*, with the fungus *B. bassiana* resulted in a treatment mortality of 70-81% at 20^0c for 1 x 10^7 conidia ml^{-1}. According to Ferron *et al*. (1991), the optimal temperatures for fungal development on the insect host range from 16-30^0c for *B. bassiana* and the *M. anisopliae* with a faster development at the higher temperatures. This was in line with the present study concerning Metarhizium isolates which resulted high percent mortality at the higher temperatures. Luz *et al*. (1998) also reported that most isolates, particularly the *B. bassiana* isolates tested against *Triatoma infestance*

proved to be more virulent at 25 and 30^0c, compared to 15 and 20^0c which was in line with the present study. Similarly, Mietkiewski *et al*. (1994) found maximal mortality in *Galleria molenella* treated with *M. anisopliae* at 30^0c.

Unlike the present study, Luz *et al*. (1998) reported two of the *M. anisopliae* isolates tested against *T. infestance* were not more virulent enough at 30^0c compared to lower temperature. Similarly, Moorhouse *et al*. (1994) reported a *M. anisopliae* isolates with highest virulence against the vine weevil, *Ostiorhynchus sulcatus*, at 10^0c whereas another isolate with an optimum at 25^0c was also reported. In the present experiment, particularly the Metarhizium isolates required higher temperature for causing maximum percent mortality than lower temperature.

The entomopathogenic fungi used in this study were isolated from different hosts and geographic origins. The result prevailed that the aphids were susceptible differently for all of the fungal isolates at different temperatures and concentrations. Unlike the present study, many authors agree that isolates of entomopathogenic fungi are generally more pathogenic to the species of insect from which they are obtained or to closely related species (Poprawski *et al*., 1985 and Maniania, 1992).

However, there are also many investigators in agreement with the present study who reported that the pathogenicity is not always related to the original insect or geographic origins (Moorhouse *et al*., 1993). Vestergaard *et al*. (1995) found that *Verticillium lecanii* isolated from thrips was weakly pathogenic to *Frankliniella occidentalis* (Pergande);

while Ekesi *et al*. (1998) reported that *B. bassiana* isolated from *Megalurothrips sjostedti* was only moderately pathogenic to its original host. Feng and Johnson (1990) also noted that the original host has no significant influence on the virulence.

Among the fungal isolates used in the present study, the lethal concentration sufficient to kill 50% of the treated insects (LC_{50}) ranged from (6.78×10^5 - 2.93×10^6), (6.89×10^5 - 9.83×10^6 conidia ml^{-1}) and (5.68×10^6 - 5.05×10^7 conidia ml^{-1}) at 30, 25 and 20^0c, respectively. At all temperature ranges, the Beauveria isolate had the lowest median lethal concentration value compared with the two Metarhizium isolates. All the Beauveria isolates at 20^0c and three of them at 25 and 30^0c had shown better virulence against adult *A. gossypii* compared with the Metarhizium isolates. Differences in pathogenicity between fungal species and isolates have also been reported for other insect species (Moorhouse *et al*., 1993; Poprawski *et al*., 1985).

In difference with the present study, different authors reported that the lower LC_{50} value was obtained for the Metarhizium isolates tested against different insect pests as compared with Beauveria isolates. Ihara *et al*. (2001) reported that the LC_{50} values of the *M. anisopliae* isolates were some what lower than those of the *B. bassiana* isolates, which indicates that the pathogenicity of *M. anisopliae* against the sting bug was higher than that of *B. bassiana*. Similarly Adane *et al*. (2002) reported that among the native fungal isolates collected from Ethiopia the Metarhizium isolate showed the lowest LC_{50} value followed by the Beauveria isolate tested against *Sitophilus zeamais*. However, Ekesi (2001) reported, in line with the present study, with regard to dose-mortality

response that one isolate of *B. bassiana* had a significantly lower LC_{50} value than the Metarhizium anisopliae isolates. In agreement with the present experiment Luz *et al*. (1998) reported the lowest LC_{50} value for a *B. bassiana* isolate tested against *Triatoma infestance* compared with *M anisopliae.*

In the present study, the median lethal time versus temperature and conidial suspension among the different fungal isolates showed some variability. For 1 x 10^8 conidia ml^{-1} at 30^0c, all fungal isolates had shown a median lethal time less than 4 day except one Beauveria isolate, DLCO 43. In this study, when temperatures and concentration were reduced, the time required to kill 50% of the treated insects was prolonged for the Metarhizium isolates compared with the Beauveria isolates.

In line with the present study, Yeo *et al*. (2003) reported that three isolates they tested in bioassays against two aphid species, *V. lecanii*, *B. bassiana* and Paecilomyces spp. showed a larger LT_{50} value at the lower temperature, 10^0c than those at 18 and 23^0c and they reported that the rate of aphid mortality due to the isolates become faster at higher temperature. In agreement with the present study, Kim *et al*. (2001) noted that the time intervals required for a mortality rate of 50% (LT_{50}) among the *A. gossypii* was lower at 20^0c than at 25 and 30^0c. They also reported that concentrations of 1 x 10^4-1 x 10^7 conidia ml^{-1} gave relatively low target mortality.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Fungal isolates of Metarhizium and Beauveria differed greatly in their capability to grow and sporulate *invitro*. Effects of isolates of Metarhizium and Beauveria on *invitro* growth and sporulation were significant over 12 days post-treatment. The interactions between tested fungal isolates with temperature and artificial culture media were significant, indicating different germination, radial growth and sporulation patterns between the Metarhizium and Beauveria isolates.

Median lethal concentration (LC_{50}) of tested fungal isolates to *Aphis gossypii* were quantified, indicating that some of the fungal isolates of Beauveria had the greatest effect on virulence as compared with the standard check. Among six fungal isolates tested, the Beauveria isolates particularly isolate DLCO 87 at both 20 and 30^0c and DLCO 41 at 25^0c had the lowest LC_{50} value as compared with the standard check ICIPE 30.

6.2 Recommendations

This research study supports the hypothesis that fungal characteristics, other than high virulence, such as germination, radial growth, sporulation and temperature tolerance, can determine the potential success of Metarhizium and Beauveria isolates for controlling *A. gossypii*. The highest virulence was obtained for the Beauveria isolates which seems better adapted in producing high mortality and coping with high temperature.

However, it is clear that further research is necessary to elucidate all of the interactions between *Aphis gossypii* and fungal pathogens. Their further development and implementation for practical field application will require improvements in the production and formulation of the pathogens; better understanding of how they will fit into integrated systems; greater appreciation for their full advantages such as efficacy, safety and selectivity.

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APPENDICES

Appendix 1. Isolates of Beauveria and Metarhizium selected from the preliminary test based on percent Mortality and speed of germination for further study against *Aphis gossypii*

No	Isolate code	Genera	Host Source	Area of collection	Mortality (%)	Germination (%)
1	DLCO 105	Beauveria	Grasshopper	Shella	76.5	45
2	DLCO 87	Beauveria	Grasshopper	Fura	88.7	58
3	DLCO 43	Beauveria	Grasshopper	Wikro	85	75
4	DLCO 41	Beauveria	Grasshopper	Erer	86.4	48
5	MM	Metarhizium	-	Arbaminch	77.5	78
6	ICIPE 30	Metarhizium	Tsetse	Kenya	83.7	85

Appendix 2. Isolates of Beauveria and Metarhizium used in the preliminary test against *Aphis gossypii*

No.	Isolate code	Genera	Host (Source)	Area of collection	Mortality (%) After 5 day
1	DLCO 2	Beauveria	Grasshopper	Burka	73.5
2	DLCO 7	Beauveria	Sorghum chafer	Showa Robit	77.5
3	DLCO 8	Beauveria	Sorghum chafer	Showa Robit	72.5
4	DLCO 11	Beauveria	Grasshopper	Bededo	74.3
5	DLCO 12	Beauveria	Grasshopper	Bededo	72.5
6	DLCO 14	Beauveria	Grasshopper	Bededo	78.3
7	DLCO 23A	Beauveria	Grasshopper	Jara	69.5
8	DLCO 34	Beauveria	Grasshopper	Qoriso	76.5
9	DLCO 35	Beauveria	Grasshopper	Artishek	77.5
10	DLCO 37	Beauveria	Grasshopper	Jara	73.5
11	DLCO 38	Beauveria	Grasshopper	Qoriso	68.5
12	DLCO 41	Beauveria	Grasshopper	Erer	86.4
13	DLCO 43	Beauveria	Grasshopper	Wikro	85
14	DLCO 44	Beauveria	Grasshopper	Wikro	74.6
15	DLCO 45	Beauveria	Grasshopper	Kobo	68.5
16	DLCO 46	Beauveria	Grasshopper	Maytimket	67.5
17	DLCO 48	Beauveria	Grasshopper	Wikro	66.7
18	DLCO 52	Beauveria	Grasshopper	Mytimket	73.5

Cont'd

19	DLCO 56	Beauveria	Grasshopper	Fura	79.8
20	DLCO 57	Beauveria	Grasshopper	Maytimket	69.8
21	DLCO 58	Beauveria	Grasshopper	Fura	77.7
22	DLCO 62	Beauveria	Grasshopper	Fura	79.4
23	DLCO 67	Beauveria	Grasshopper	Fura	78.5
24	DLCO 74	Beauveria	Grasshopper	Kobo	68.5
25	DLCO 75	Beauveria	Grasshopper	Maytimket	76.5
26	DLCO 78	Beauveria	Grasshopper	Maytimket	77.3
27	DLCO 82	Beauveria	Grasshopper	Erer	76.3
28	DLCO 84	Beauveria	Grasshopper	Fura	79.6
29	DLCO 85	Beauveria	Grasshopper	Debre-Zeit	65.5
30	DLCO 86	Beauveria	Grasshopper	Fura	74.5
31	DLCO 87	Beauveria	Grasshopper	Fura	88.7
32	DLCO 88	Beauveria	Grasshopper	Qoriso	75.7
33	DLCO 95	Beauveria	Grasshopper	Shella	66.5
34	DLCO 96	Beauveria	Grasshopper	Debre-Zeit	62.5
35	DLCO 98	Beauveria	Grasshopper	Arbaminch	67.5
36	DLCO 99	Beauveria	Grasshopper	Arbaminch	61.5
37	DLCO 100	Beauveria	Grasshopper	Shella	63.5
38	DLCO 105	Beauveria	Grasshopper	Shella	76.5
39	DLCO 107	Beauveria	Grasshopper	Arbaminch	75.7
40	DLCO 116	Beauveria	Grasshopper	Minjar	78.7

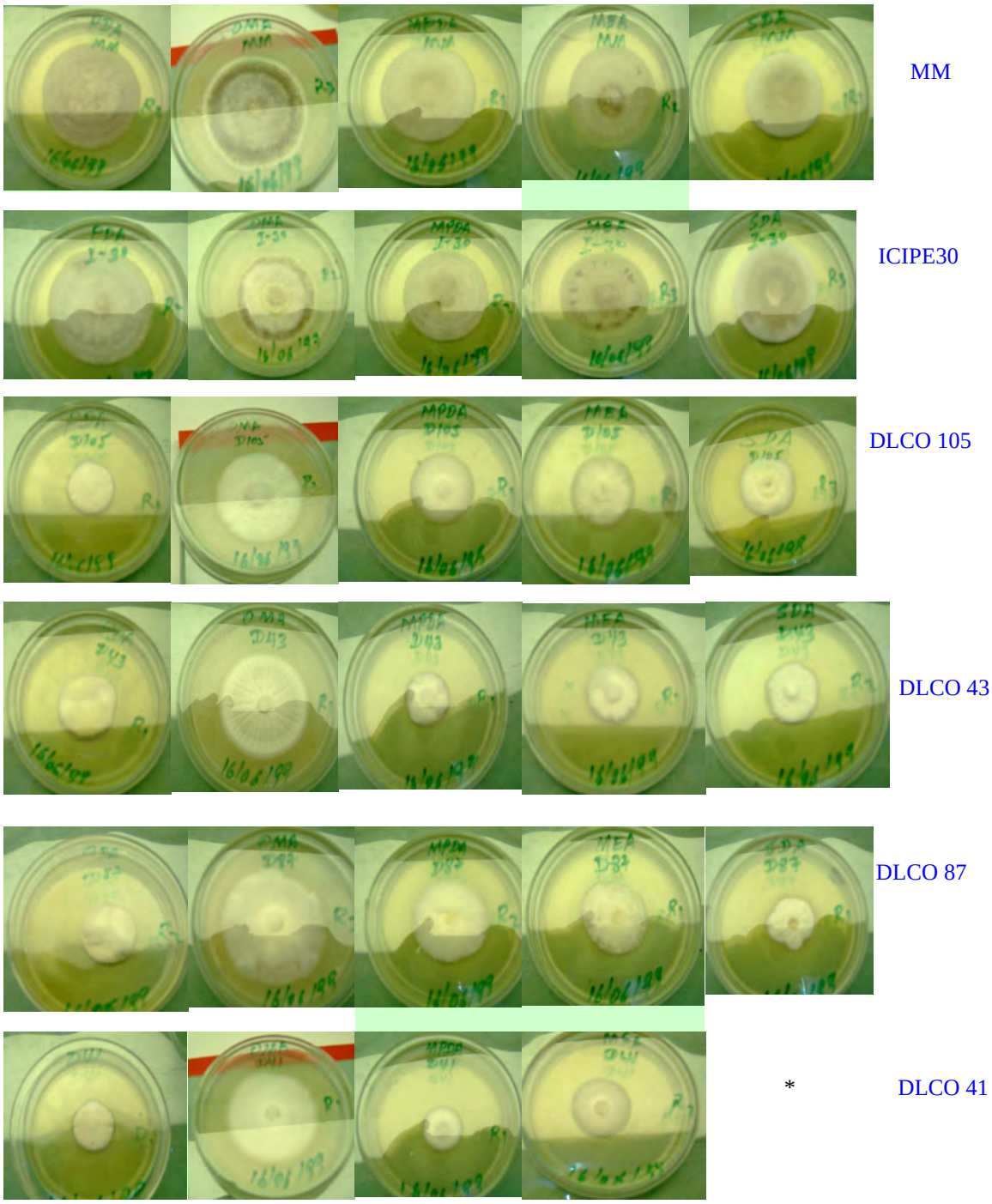
Cont'd

41	DLCO 119	Beauveria	Grasshopper	Chichu	60.5
42	DLCO 120	Beauveria	Grasshopper	Alaba	59.5
43	DLCO 121	Beauveria	Grasshopper	Ziway	67.5
44	DLCO 124	Beauveria	Grasshopper	Debre-Zeit	63.5
45	DZ	Beauveria	Grasshopper	Debre-Zeit	73.5
46	PPRC 56	Beauveria	Pachnoda	Berber	71.5
47	PPRC 9609	Beauveria	Mugundo	Blosyrus	61.3
48	DLCO 28	Metarhizium	Grasshopper	Biyo	64.5
49	DLCO 32	Metarhizium	Grasshopper	Jara	66.5
50	DLCO 50	Metarhizium	Grasshopper	Adele	65.6
51	DLCO 51	Metarhizium	Grasshopper	Adet	67.8
52	DLCO 63	Metarhizium	Grasshopper	Kobo	63.3
53	DLCO 91	Metarhizium	Grasshopper	Debre-Zeit	69.5
54	DLCO 115	Metarhizium	Grasshopper	Ziway	65.5
55	EE	Metarhizium	Crustacean	Alamata	70.5
56	MA	Metarhizium	Soil	Arbaminch	71.3
57	MM	Metarhizium		Arbaminch	77.5
58	PPRC 6	Metarhizium	Pachnoda	Kewot	62.5
59	ICIPE 30 (Standard check)	Metarhizium	Tsetse	Kenya	83.7

Appendix 3. Speed of germination of Metarhizium and Beauveria isolates selected from those having greater than or equal to 75 % mortality in the preliminary screening test

No.	Isolate code	Genera	Mortality (%)	Germination (%) After 12 h
1	DLCO 7	Beauveria	77.5	32
2	DLCO 14	Beauveria	78.3	35
3	DLCO 41	Beauveria	86.4	48
4	DLCO 43	Beauveria	85	75
5	DLCO 58	Beauveria	77.7	27
6	DLCO 82	Beauveria	76.3	35
7	DLCO 84	Beauveria	79.6	28
8	DLCO 87	Beauveria	88.7	58
9	DLCO 105	Beauveria	76.5	45
10	DLCO 116	Beauveria	78.7	32
11	ICIPE 30	Metarhizium	83.7	85
12	MM	Metarhizium	77.5	78
13	DLCO 107	Beauveria	75.7	39
14	DLCO 12	Beauveria	76.6	40
15	DLCO 67	Beauveria	78.5	34
16	DLCO 88	Beauveria	75.7	29
17	DLCO 78	Beauveria	77.3	36
18	DLCO 56	Beauveria	79.8	38
19	DLCO 75	Beauveria	76.5	41
20	DLCO 35	Beauveria	77.5	39
21	DLCO 34	Beauveria	76.5	37
22	DLCO 62	Beauveria	79.4	35

h = hours



* Photo was missed for DLCO 41 on SDA medium

Appendix **4**. Plates of the influence of different solid culture media on radial growth of Beauveria and Metarhizium isolates

Dry sterilizer

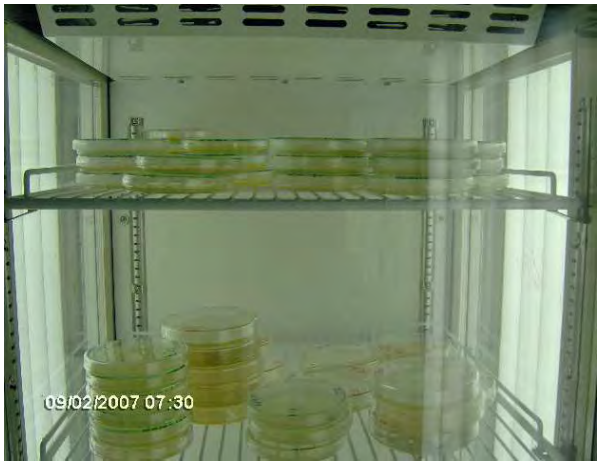
Steam sterilizer

Vortexer

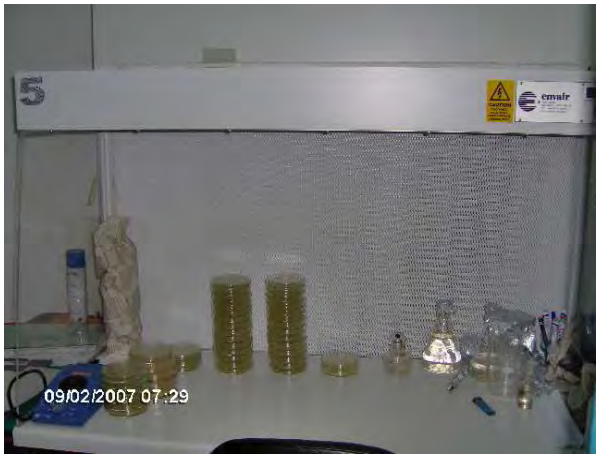
Micropipette

Microscope

Cork borer and Sprayer



Incubator



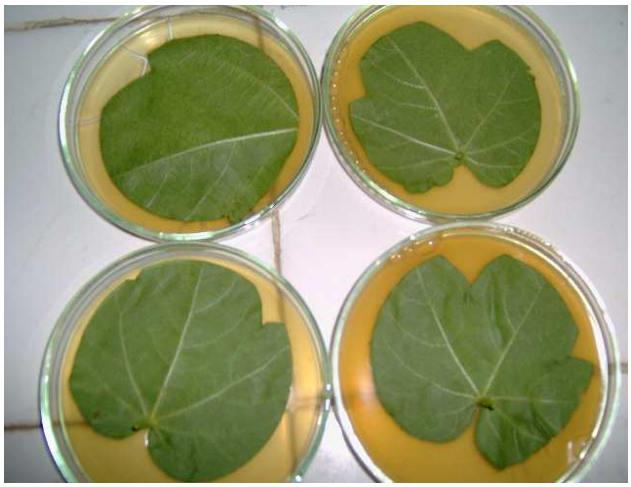
Laminar flow cabinet

Appendix 5. Important laboratory equipments used for the experiment

Media preparation and water agar

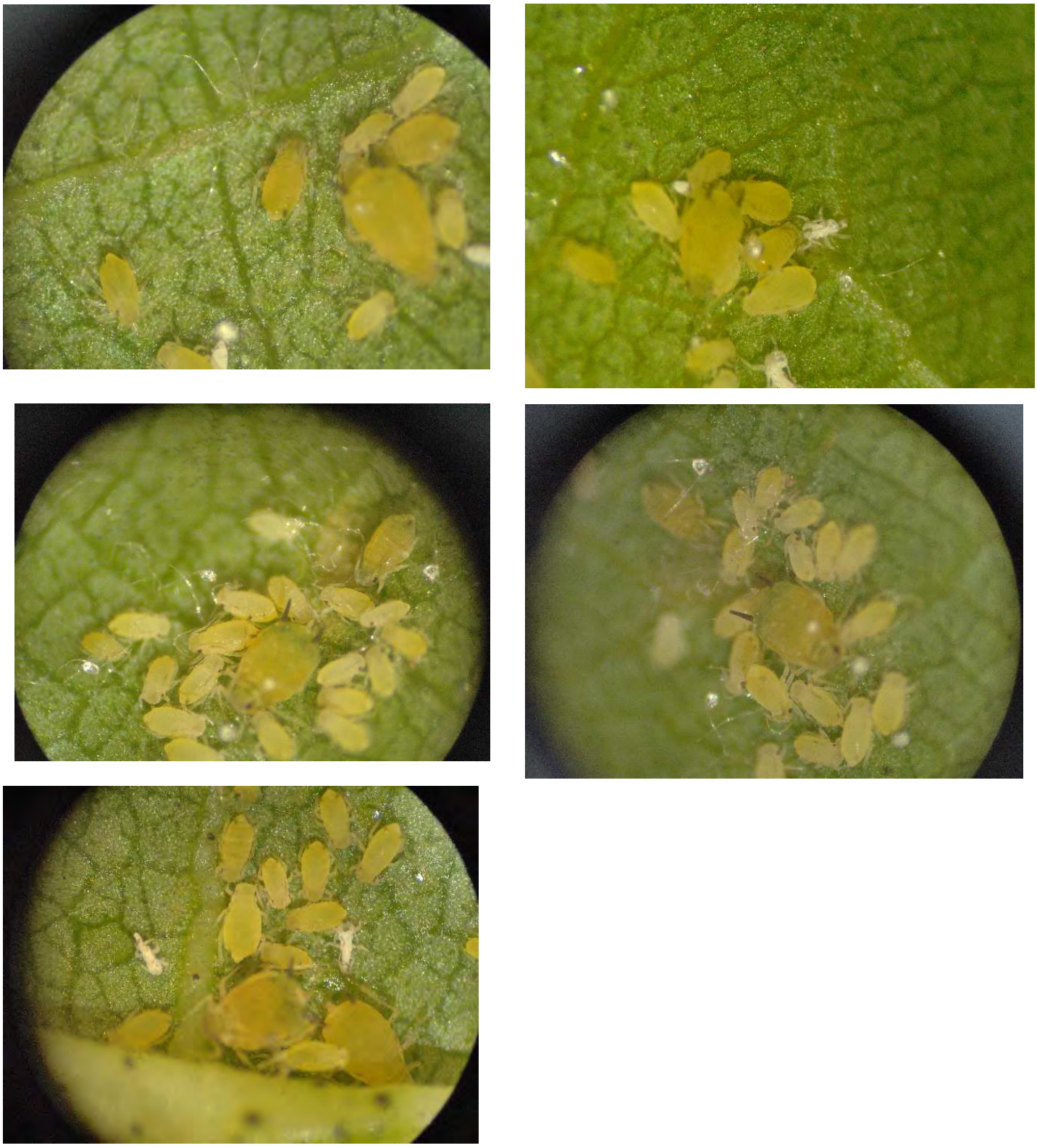
Cotton leaves

Cotton leaf discs



Water agar embedded cotton leaf

Appendix **6**. Preparation of nutrient media, cotton leaf disc and water agar embedded cotton leaf



Appendix 7. Laboratory rearing of *Aphis gossypii*