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ARTICLE

In-vitro pathogenicity of *Akanthomyces lecanii* and *Metarhizium anisopliae* against the aphid *Aphis craccivora*

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Resumen

Patogenicidad in vitro *de* Akanthomyces lecanii *y* Metarhizium anisopliae *contra el pulgón* Aphis craccivora

Aphis craccivora es una plaga mundial grave de la alubia ojo de perdiz y responsable del bajo rendimiento del cultivo. Los hongos entomopatógenos ofrecen alternativas ambientalmente respetuosas a pesticidas sintéticos convencionales. Se evaluó el potencial de *Akanthomyces lecanii* y *Metarhizium anisopliae* contra el pulgón negro de las leguminosas en laboratorio. Estos hongos se utilizaron en bioensayos de laboratorio: Se rociaron discos de papel con diferentes concentraciones de esporas de cada aislado, con hojas de judía como alimento para los insectos. Se observó y registró la mortalidad de los pulgones durante 10 días. La concentración de $1x10^8$ conidios/ml fue suficientemente alta para causar mortalidad en todos los ensayos, mientras que en el control fue del 10%. Este estudio confirma el potencial de hongos autóctonos como agentes de control biológico contra estos pulgones, incluso a bajas concentraciones.

Palabras clave: Hongos entomopatógenos; Bioensayo de patogeneidad; Pulgón negro de las leguminosas; Control biológico.

Abstract

Aphis craccivora is a serious pest of cowpea worldwide and responsible for low crop yields. Entomopathogenic fungi offer environmentally friendly alternatives to conventional synthetic pesticides. In the present study, the biological control potential of *Akanthomyces lecanii* and *Metarhizium anisopliae* against cowpea aphid was evaluated under laboratory conditions. These fungi were used in the laboratory bioassays: Conidial suspensions with different concentrations of spores of each isolate were sprayed on filter-paper discs on which bean leaves were placed as food for the insects. Aphid mortality was observed and recorded for 10 days. The concentration of $1x10^8$ conidia/ml was high enough to cause insect mortality in all the isolates tested while the control mortality was 10%. This study confirms the potential of using the indigenous fungi as biological control agents against the cowpea aphids even at low concentrations.

Key words: Entomopathogenic fungus; Pathogenicity bioassay, Cowpea aphid; Biological control.

Introduction

Aphids (Hemiptera: Aphididae) are cosmopolitan species that have been identified amongst the major insect pest in tropical and temperate regions (Vasanthara & Kumarswami 1982). *Aphis craccivora* Koch, 1854, the cowpea aphid, are known to be highly polyphagous succivorous and feed on an important amount of crops (as it is recorded feeding on plants belonging to eight plant families) since aphids are adapted to transmit different viruses when they suck the sap. They also live in symbiotic association with ants, and in turn, they protect them from natural enemies and transport them from one place to another, at the same time that they take from them the sugary excrement, known as dew of honey (Simbaqueba *et al.* 2014, Namitha *et al.* 2021). *Aphis craccivora* is the black aphid and is the main pest of cowpea bean reported in regions of Africa, Asia and Latin America (Pettersson *et al.* 1998) quoted by (Obopile & Ositile 2010, De La Pava & Sepúlveda-Cano 2015).

Cowpea aphid is polymorphic (with apterous and alate form), viviparous and in the tropics parthenogenetic reproduction occurs throughout the year, and it is difficult to manage partly due to its polyphagous nature with very short life cycle (like for example 13 days) and high reproduction rate and adults live approximately 6 to 5 days (Obopile & Ositile 2010, Jaramillo-Naranjo 2015, Namitha *et al.* 2021). This is an ovoviviparous insect, the female adults nurture the egg in its body before it is hatched into larvae (fundatrix) which subsequently passed through four instar nymph stages before reaching adult stage (Obopile & Ositile 2010). The nymphs are composed of dark brown/dull grey and wingless body with some deposition of wax. Matured adults possess wings, usually darker and shiny with no wax deposition as in the nymph (Obopile & Ositile 2010). All the growth stages possess white and black legs and with the distal part of femur, siphunculi and cauda being black (Obopile & Ositile 2010, Latinović *et al.* 2017). The antennae usually six segmented. In general, adults are mostly shiny black or dark brown in color, variable in size between 1.5 and 2 mm long. The nymphs are wingless, dark brown in color and round in shape (Obopile & Ositile 2010, De la Pava & Sepúlveda-Cano 2015).

It is a serious pest of leguminous crop and sucks the sap from tender shoots, inflorescence and pods resulting in the drying up of tender shoot and premature fall of flower buds, flowers and tender pods. Crops such as cowpea, field bean, groundnut, chickpea, mung bean, urd bean, pigeon pea, brassicas, cucurbits, beetroot, and cotton, have all been reported to be attacked by this aphid (Namitha *et al.* 2021). The honey dew secretion of the aphids provides a suitable media for the development of sooty mould and fungi which ultimately hampers the process of photosynthesis (Obopile & Ositile 2010, Selvaraj & Kaushik 2014). However, this aphid has been implicated as a vector of many plant viruses such as rosette, mottle, stunt and stripe (Kokalis-Burelle *et al.* 1984).

The cowpea aphid is an important pest of the crop that has led to yield losses of about 20-100% (Obopile 2006). Effect of *A. craccivora* on cowpea may include direct and indirect damage through sucking of plant sap and transmission of different plant viruses (Blackman & Eastop 2000). Indiscriminate use of synthetic pesticides has resulted in many conspicuous problems including pesticide resistance, disruption of beneficial fauna and other environmental and human health issues. (Asi *et al.* 2009, Nazir *et al.* 2019, Iqbal *et al.* 2021, Aliyu & Fidelis 2021). To overcome these problems associated with the extensive use of synthetic pesticides on pests, alternative approaches such as the use of biological control agents have been widely studied in many countries (Motta-Delgado & Murcia-Ordoñez 2011, Mora *et al.* 2017).

Several strategies have been deployed over the years to control the menace of aphids. The biological control of aphids by natural enemies has become an important component of pesticide-free management strategies (Zehnder *et al.* 2007). Natural enemies of aphids include parasitic wasps, coccinelids which are primarily aphidophagous, and generalist predators (such as spiders and ground beetles) that frequently feed on other prey in addition to aphids (Desneux *et al.* 2009, Dixon 2000, Birkhofer & Wolters 2012). However, the effectiveness and sustainability of these predators are associated with considerable uncertainties, since the strength of predator effects on pest numbers depends on a range of external factors (Diehl *et al.* 2013).

The use of entomopathogenic fungi as part of integrated pest management program (IPM) is a

technology that has advanced in recent times (Lacey *et al.* 2015). This is because they can be used to control a wide variety of agricultural pests (Fernández-Grandon *et al.* 2020). They serve as alternatives to harmful synthetic pesticides due their ecologically friendly nature as they are safe to humans, farm animals and most of all they do not pollute the environment (Sinha *et al.* 2016). They have specific hosts and their mode of action is known. The entomopathogenic fungi usually produce spores/conidia which attach to the cuticle of their hosts (Fernández-Grandon *et al.* 2020). Subsequently, they penetrate and initiate an infection in susceptible hosts at high humidity (Amnuaykanjanasin *et al.* 2013, Fernández-Grandon *et al.* 2020), but the spores can remain viable on the cuticle when the conditions are unfavourable and on return of favourable conditions can initiate an infection (Fernández-Grandon *et al.* 2020). As with other entomopathogenic fungi, the process of aphid colonization by *Akanthomyces lecanii* (Zimm.) Spatafora, Kepler & B. Shrestha (=*Verticillum lecanii*) involves a sequence of events including spore attachment, germination, cuticle penetration, and active multiplication in host tissues (Fournier and Brodeur 2000). The adhesion: The surface structure and composition of the insect exosqueleton influence the adherence of fungal conidia to the cuticle. The outhermost layer of the insect integument is a lipid layer, which is hydrophobic in nature, facilitating attachment of fungal propagules. The cuticular penetration: The conidial surface proteins act synergistically to aid in germination through recognition of insect-specific components and subsequent cuticle degradation. Once the fungal conidia successfully adhere to the insect cuticle, they germinate to form hyphae on the insect cuticle and express hydrolytic enzymes, such as proteases, esterases, N-acetylglucosaminidases, chitinases and lipases. In addition to enzymatic degradation, mechanical pressure through formation of specialized hyphal structures (appresoria) has also been implicated for successful cuticular penetration. Proliferation, immune avoidance, and insect death: Through the combination of mechanical pressure and enzymatic processes, fungal hyphae penetrate the insect cuticle and eventually reach the insect hemocoel, where they differentiate to form yeast-like bodies called blastospores. Insect hemolymph is rich in nutrients (being mainly the trehalose). Upon reaching the insect hemolymph, the fungal hyphae switch phenotypes to blastospores and short hyphal lengths called hyphal bodies. Successful penetration of the fungus is accompanied by its multiplication and colonization of the internal organs of the insect host. Entomopathogenic fungi to avoid being detected by the host's immune system, such as *Metarhizium* Sorokīn expresses a collagen-like protein (mcl 1) which functions as a defensive coat that prevents hyphal bodies from being phagocytosed or encapsulated by hosts immune cells.

Conidiation on the surface of the insect cadaver

The blastospores proliferating within the hemolymph kill the insect host within 3-7 days by absorbing hemocoelic nutrients and through toxic metabolite production. After fungal hyphae ramify throughout the dead infected host, they reemerge from the insect and conidiate on the insect cadaver (Wang *et al.* 2016).

Following death of insect host, hyphae production by the entomopathogenic fungi is usually accompanied by the production of numerous spores/ conidia on the host cadaver (Fernández-Grandon *et al.* 2020). Spores produced in this way will disperse and infect more susceptible hosts on the farm conferring a great advantage of using entomopathogenic fungi for pest management on the farms (Fernández-Grandon *et al.* 2020). Entomopathogens are well characterized in respect to pathogenicity against several insect pests and have been used as mycoinsecticides for the biological control of agricultural pests worldwide (Sandhu *et al.* 2012). A major advantage of using entomopathogenic fungi in insect pest control is that these fungi can infect all stages of the insects, ranging from larval and adult stages of development (Butt *et al.* 2016).

A variety of entomopathogenic fungi have been exploited worldwide for the biological control of important insect pests of agricultural produce. This is because these important entomopathogens are highly effective (Gurlek *et al.* 2018), environmentally friendly and target-specific (Gebremariam *et al.* 2021, Santos *et al.* 2021). Several mycoinsecticides based on *Beauveria bassiana* (Bals.-Criv.) Vuill., *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (=*Paecilomyces fumsoroseus*), *Akanthomyces lecanii* (Zimmermann) Viegas (=*Lecanicillium lecanii*)

and *Metarhizium anisopliae* (Metschn.) Sorokīn have been employed in the control of several insect pests of agricultural produce (Upadhyay *et al.* 2014, Barra-Buarei *et al.* 2016, Aliyu *et al.* 2022). Fungal pathogens occur very widely in nature and there is a wide scope for isolating strains of fungal pathogens with enhanced virulence as well as desired cultural characteristics (Rabindra & Ramanujam 2007).

Entomopathogenic fungi are pathogenic to various pests and can be used as biological control agents by alternatively replacing chemical pesticides for cowpea aphid management; however, entomopathogenic fungi in aphids have been studied in cotton aphid pests *Aphis gossypii* Glover, 1877. The conidia of entomopathogenic fungi inv *M. anisopliae* ade the aphid by attaching to the epidermis. Entomopathogenic fungi kill insects by secreting secondary metabolites that act as toxins. *Beauveria bassiana* is known to secrete beauvericin, bassianin, bassianolide, and oosporein after invading insects. Of the fungal species, *B. bassiana* and *M. anisopliae* exhibit significantly high virulence against *A. gossypii*. *B. bassiana* is also pathogenic to other aphids including *A. craccivora*, *Sitobion avenae* (Fabricius, 1775), the spike aphid: oats, rye, barley and corn; *Schizaphis graminum* (Rondani, 1852), the cereal aphid; *Rhopalosiphum padi* (L., 1758), the oat aphid; Brevicoryne brassicae (L., 1758), the broccoli aphid, and Lipaphis erysimi (Kaltenbach, 1843) (= *Hyadaphis pseudobrassicae*), the turnip aphid in America and other cruciferous crops in India. RNA sequencing showed that Conidiobolus obscurus (I.M.Hall & P.H.Dunn) Remaud. & S.Keller (Zygomycetes: Entomophthorales), a fungal pathogen of cereal aphids, overexpresses the cytolytic-like δ-endotoxin gene and serine proteases while invading and killing aphids. In addition, *A. lecanii* is known to increase pathogenicity against aphids by producing an enzyme that hydrolyzes aphid chitin through Vlchit1 expression (Im *et al.* 2022). In addition, it was found that the pathogenicity of both *Purpureocillium lilacinum* (Thom) Luangsaard *et al.* (=*Paecilomyces lilacinus*) and *B. bassiana* strains used in the experiments against cotton aphids negatively affected aphid reproduction over periods of seven and 14 days in a series of greenhouse trials and in field trials the plants inoculated with *B. bassiana* had significantly lower numbers of aphids and the number of aphids on plants inoculated with *P. lilacinum* exhibited a similar, but non-significant, reduction in numbers relative to control plants. Also tested pathogenicity of both *P. lilacinum* and *B. bassiana* strains used in the experiments against cotton aphids in a survival experiment where 60% and 57% of treated aphids, respectively, died from infection over seven days versus 10% mortality among control insects (Castillo-López *et al.* 2014).

Several commercial formulations based on entomopathogenic fungi were developed for the control of sucking pests in different countries. Mycotrol and Botanigard based on *B. bassiana*, Mycotal based on *A. lecanii* and PFR-97 and PaeSin based on *Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm. were developed for the control of whiteflies, aphids and thrips in USA, Europe and Brazil. In India, *Fusarium pallidoroseum* (Cooke) Sacc. was found effective in controlling cowpea aphid in Kerala (Rabindra & Ramanujam 2007).

Díaz *et al.* (2008) reported Entomophthorales fungi as important antagonists of aphids, causing natural epizootics capable of drastically reducing their populations. The development of these epizootics (a disease that temporarily predominates in a region or locality and simultaneously attacks a large number of individuals of one or several animal species) is facilitated by a series of morphological (soft body and small size) and biological characteristics. (short life cycle, often parthenogenetic, viviparous, the apterous and winged forms of the adult), typical of aphids that favor the transmission of fungi between individuals in a population and the environment where they live. Therefore, entomopathogenic fungi are interesting as biocontrol agents within the biological control of aphids.

Between the fungi, entomophthoralean fungi (Zygomycetes: Entomophthorales) like Erynia neoaphidis Remaud. & Hennebert, *Neozygites fresenii* (Nowak.) Remaud. & S. Keller and *Zoophthora radicans* (Brefeld) Batko were reported to cause epizootics in several aphid species in nature (Rabindra & Ramanujam 2007). In addition, there is a study of entomophthoralean fungi causing infections in natural populations on alfalfa aphids (*Medicago sativa* L.) in the province of Santa Fe (Argentina), where they found four species of entomophthoroid fungi, *Pandora neoaphidis* (Remaudiére y Hennebert) Humber, *Z. radicans*, *Entomophthora planchoniana* Cornu and *N. fre-*

senii infecting *A. craccivora*, *Therioaphis trifolii* (Monell, 1882), the spotted alfalfa aphid, and *Acyrthosiphon pisum* (Harris, 1776), the pea aphid, and unidentified species of *Acyrthosiphon* Mordvilko, 1914. In this study, *Z. radicans* was the most important pathogen, recorded mainly on *Acyrthosiphon* sp. and successfully isolated and maintained in pure cultures (Manfrino *et al.* 2014).

In view of the ecological and environmental stress associated with the use of synthetic chemical insecticides and the current need to develop and use eco-friendly alternatives of biological control, this study is carried out to evaluate the pathogenicity of *A. lecanii* and *M. anisopliae* against cowpea aphids under laboratory conditions.

Materials and methods

Insects

Large number of cowpea aphid were collected from farms within Bauchi metropolis (Bauchi State) with the aid of a sweep net. The insects were brought to the Ecology laboratory of Abubakar Tafawa Balewa University for rearing and maintain a laboratory stock. A stock culture of the insect was maintained on broad bean plant, *Phaseolus vulgaris* L., under laboratory conditions of 22 ± 7 ºC and 60 ± 7% relative humidity for several generations. At every experiment, the insects were put on fresh tender bean leaves cultivated in small, ventilated containers (10 cm in diameter, one plant/container).

Fungal isolates

Akanthomyces lecanii and *M. anisopliae* isolates were obtained from fungi stock culture collection of the Ecology laboratory of Abubakar Tafawa Balewa University, (ATBU) Bauchi and maintained on potato-dextrose agar (PDA) plates. These fungi are local isolates that have been maintained in the laboratory over some years while their isolation methods and morphological characterization had been described previously (Yakubu *et al.* 2022).

Conidial Suspensions Preparation

The conidia were harvested from 14-day old surface cultures by scraping and weighing 0.1g of the culture in a test tube with 9 ml of distilled water containing 0.01% Tween® 80 (Sigma USA). Serial dilutions of 1×108, 1× 107 and 1x106 conidia/ml were prepared. The concentrations of conidia were determined using a Neubauer hemocytometer at 400x magnification (Olympus BX23, Tokyo, Japan).

Pathogenicity of fungal strains against *A. craccivora*

Two mililiters of the conidial suspension for each of the dilutions from each isolate was sprayed on filter-paper discs (diameter 9 cm) placed in a vial and sterilized broad bean leaves were placed in the vials as feed for the insects. 10 adult aphids were placed in each of the vials and each treatment was replicated in triplicate (that is n = 30 per treatment). The broad been leaves were sterilized with 1% sodium hypochlorite and rinsed three times with distilled water for approximately 3 min and allowed to dry in a sterile incubator.

The vials were covered with a cotton mesh for air to circulate. The control was processed as described above, except that conidial suspension was replaced with 0.01% Tween® 80 water solution. Mortality of the aphids was observed and recorded daily for 10 days (Fournier and Brodeur 2000).

Dead insects were removed from the vial and kept in the dark at 90% relative humidity to promote fungal development and sporulation in order to confirm that the insects died due to infection by tested fungal strain (Keyser *et al.* 2016).

Data Analysis

Mortality data was corrected with that in control by using the Abbott's formula (Abbott 1925), while the per cent corrected cumulative mortality of each fungus was compared using Mantel-Cox Log-rank test. The survival curve was plotted against time and concentration for each fungus. The median lethal concentration (LC_{50}) and the median lethal time (LT_{50}) values were computed by using Graphpad Prism 8 and were compared between the two fungi species using the two sample t-test procedure.

Results

Pathogenicity of fungal strains against *A. craccivora*

The results of pathogenicity showed that both fun-

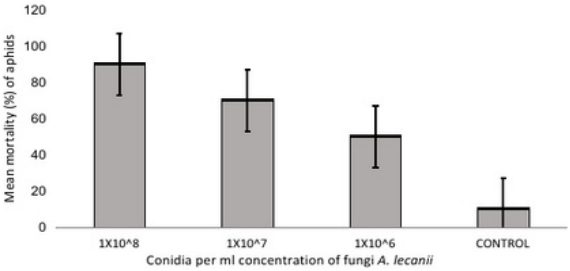

Figura 1. Mortalidad media (%) de *A. craccivora* a diferentes concentraciones de conidios del hongo entomopatógeno *A. lecanii*.

Figure 1. Mean mortality (%) of *A. craccivora* at different concentrations of conidia of fungi entomopathogenic *A. lecanii*.

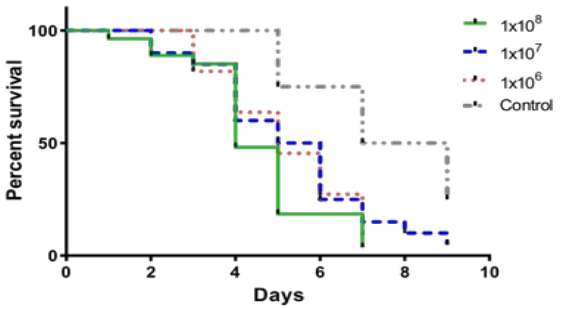

Figura 2. Curva de supervivencia de *A. craccivora* frente a la patogeneidad de *A. lecanii*.

Figure 2. Survival curve of *A. craccivora* to pathogenicity of *A. lecanii*.

Mantel-Cox Log-rank test for survival comparison of the aphids to the fungi treatments. 95% confidence level, n = 30. * p<0.05.

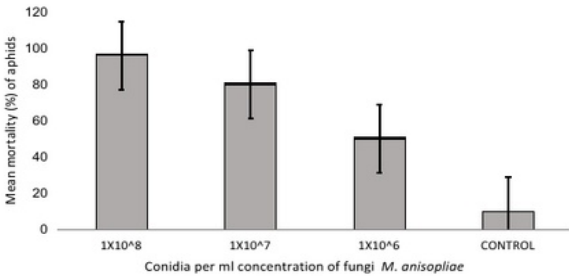

Figura 3. Mortalidad media (%) de *A. craccivora* a diferentes concentraciones de conidios de *M. anisopliae*.

Figure 3. Mean mortality (%) of *A. craccivora* at different concentrations of conidia of *M. anisopliae*.

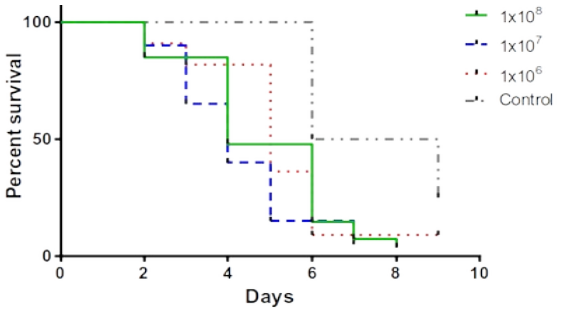

Figura 4. Curva de supervivencia de *A. craccivora* frente a la patogeneidad de *M. anisopliae*.

Figure 4. Survival curve of *A. craccivora* to pathogenicity of *M. anisopliae*.

Mantel-Cox Log-rank test for survival comparison of the aphids to the fungi treatments. 95% confidence level, n = 30. * p<0.05.

gal isolates tested produced varied mortality rate against aphids. *Akanthomyces lecanii* has percentage mortality of 90% at $1x10^8$ conidia per ml, 70% mortality at $1x10^7$ conidia per ml and 50% at $1x10^6$ conidia per ml while the control produced only 10% mortality (Fig. 1). The survival curve comparison (Mantel-cox test) showed that there was no significant difference ($p>0.05$) in the concentration (dose) response of aphids to *A. lecanii* (Fig. 2).

Metarhizium anisopliae produced a percentage mortality of 96% at $1x10^8$ conidia per ml, 80% mortality at $1x\ 10^7$ and 50% mortality at $1x10^6$ while the control produced 10% mortality (Fig. 3). The survival curve comparison (Mantel-cox test) showed that there was significant difference ($p< 0.05$) in the concentrations (doses) response of aphids to *M. anisopliae* (Fig. 4).

Lethal concentration and time lethal

Table 1 shows the LC_{50} and LT_{50} values of the two entomopathogenic fungi. Low LC_{50} value of 10^8 spores/ml for *A. lecanii* against *A. craccivora* was 2.9×10^6 spores/m and it was significantly lower to that of *M. anisopliae* (LC_{50} value of 4.2×10^7 spores/ml). The LT_{50} value at 10^8 spores/ml. from *A. lecanii* and *M. anisopliae* were 3.9 and 5.2 days respectively.

Conidiation capacity (sporulation) of *A. lecanii* and *M. anisopliae*

The entomopathogenic fungi V. lecanni began sporulating at 5 days post insect death and the fungi M. anisopliae began sporulating at 3 days post insect death.

In the figures 5 and 6, the sporulation (conidiation) of *A. lecanii* and *M. anisopliae* are recorded respectively on dead adult aphids after the bioassay period of 10 days in relation to the control (Fig. 7).

Discussion

Virulence of two indigenous entomopathogenic fungi *A. lecanii* and *M. anisopliae* was tested against an important pest of cowpea *A. craccivora* in the laboratory using three concentrations of fungal conidia. The results showed that both entomopathogenic fungi isolates were found to be virulent against the test insect pest even though *M. anisopliae* produced a higher mortality rate at

Fungi	LC_{50} (spores ml^{-1})	95% FL (spores ml^{-1})	LT_{50} (days)
V. lecanni	$2.9\times10^6 \pm 0.2\times10^6$*	1.2×10^6 - 3.8×10^6	3.9 ± 0.3*
M. anisopliae	$4.2\times10^7 \pm 1.3\times10^6$	3.8×10^6 - 7.1×10^7	5.2 ± 0.6

Tabla 1. Concentración letal y tiempo para causar el 95% de mortalidad a *A. craccivora* a concentration 1×10^8. *indica diferencias significativas ($p<0.05$). FL: límite de referencia.

Table 1. Lethal concentration and time for the isolates to cause 50% mortality to the *A. craccivora* at 1×10^8 concentration.
*indicates significant difference ($p<0.05$). FL: fiducial limit.

concentrations of $1x10^8$ and $1x10^7$ with both isolates producing the same effect of 50% at $1x10^6$. This observation is in conflict with those of Alavo *et al.* (2002) and Vestergaard *et al.* (1995) who reported the unreliability of *A. lecanii* for the control of aphid pest as compared to *M. anisopliae*.

Observations in this study shows that the virulence of entomopathogenic fungi is usually concentration dependent as concentration of $1x10^8$ produced 90% and 70% mortality for *A. lecanii* and *M. anisopliae* respectively.

Mortality rates also declined with decrease in spore concentrations of the isolates and similar observation were made by Fournier & Brodeur (2000).

Low LC_{50} value of 2.1×10^6 spores/ml for *A. lecanii* against *A. craccivora* (Abdel-Raheem *et al.* 2021) and 2.7×10^4 spores/ml against *A. gossypii* was reported by Derakshan *et al.* (2007). Abdel-Raheem *et al.* (2021) also reported 6.4×10^7 spores/ml for *M. anisopliae* against *A. craccivora* and Chandler *et al.* (1997) mentioned that for *M. anisopliae* it was $2.45x10^6$ spores/ml.

However, the variations observed in the virulence of these entomopathogenic fungi (as measured by the lethal concentration-response) to various insect pests can be attributed to both intrinsic an extrinsic factor which includes environmental factors, sporulation and concentrations of the fungi culture. Nonetheless, LT_{50} value at 10^9 spores/ml from *A. lecanii* and *M. anisopliae*, 4.2 and 7.0 days respectively has been reported by Abdel-Raheem *et al.* (2021).

Results obtained in this study also followed similar pattern of previous studies that reported the efficacy of several entomopathogenic fungi as well as *A. lecanii* and *M. anisopliae*, either singly or in association with botanical extracts for the biological control and management of different species of aphids that attack and destroy agricultu-

Figura 5. Esporulación de *A. lecanii* en áfidos adultos muertos *A. craccivora* tras el periodo de bioensayo de 10 días.

Figure 5. Sporulation (Conidiation) of *A. lecanii* on dead adult aphids *A. craccivora* after the bioassay period of 10 days.

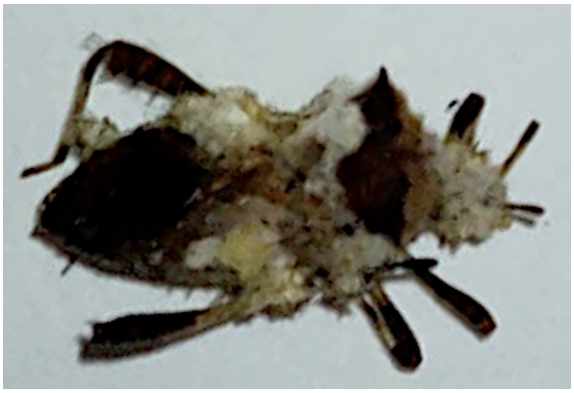

Figura 5. Esporulación de *M. anisopliae* en áfidos adultos muertos *A. craccivora* tras el periodo de bioensayo de 10 días.

Figure 5. Sporulation (Conidiation) of *M. anisopliae* on dead adult aphids *A. craccivora* after the bioassay period of 10 days.

Figura 7. Áfido adulto como control

Figure 7. Adult aphid *A. craccivora* as control.

ral crops (Fernández-Grandon *et al.* 2020, Ali *et al.* 2018, Yun *et al.* 2017). Overall, both fungi demonstrated their ability to recycle on the test pests by sporulating on the cadavers as shown in figures 5 and 6. The conidiation of entomopathogenic fungi on the surface of the insect cadaver according to Wang *et al.* (2016) express that the blastospores proliferating within the hemolymph kill the insect host within 3-7 days by absorbing hemocoelic nutrients and through toxic metabolite production. After fungal hyphae ramify throughout the dead infected host, they reemerge from the insect and conidiate on the insect cadaver. So the fungus *N. fresenii* infecting aphids, especially species of the genus *Aphis* L. 1758 and the infection mechanism of the fungus to the aphid is as follows capilloconidia adhere to the aphid by the sticky apical droplet. A germ tube is produced which forms an appressorium on the insect cuticle and a tube from the appressorium then penetrates it. Aphids killed by *N. fresenii* characteristically hang from the stems and the underside of leaves of the host plant by the proboscis inserted in the plant tissues. *Aphis fabae* Scopoli, 1763, the bean aphid, killed by this species are orange in colour when dry and grey in moist conditions as the fungus begins to sporulate. This fungus is most frequently associated with dense populations of aphids in warm seasons and is unusual in attacking aphid populations in the tropics (Wilding & Brady, 1984).

The idea of biological control based on entomopathogenic fungi is on the increase mainly due to high environmental awareness, concerns on consuming safe food and disappointments from the use of conventional synthetic pesticides that result from resistance and resurgence of pests. In the current study, the cowpea aphids were susceptible to the indigenous isolates of entomopathogenic *A. lecanii* and *M. anisopliae* and producing a mortality rate of 90% and 96% respectively. The isolates are widely distributed and amenable to mass production in the laboratory using local and cheap media. As well, the safety of the isolates for humans, the environment, non-target organisms and their non-residual effect on food makes them the best alternatives for exploitation and use as biological control agents in the management and control of this important pest of cowpea. Hence, the isolates can be safely integrated into Integrated Pest Management program for aphid control in Nigeria.

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