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Research Article

Biological Control of Locusts and Grasshoppers Using Some Fungi

Shinkafi, S.A. and Sanusi, M.M.
Department of Microbiology, Usmanu Danfodiyo University Sokoto, Nigeria

Corresponding author
Shinkafi, S.A
Email: saadatushinkafi@rocketmail.com

Abstract: Fungal strains such as *Metarhizium anisopliae, Beauveriam bassiana, Metarhizium flavoride,* and *Entomophaga grylli* associated with soil were isolated from various insects including termites, and screened for their ability to control insects such as locusts, grasshoppers, trips, termites, and Mosquitoes. Three among the strains were highly virulent against insects during caging and feeding. The cultivation of these strains by spore production indicated that three strains of *Metarhizium spp* produced more spores and remains viable at relatively low humidity and temperature of 40"C. The three fungal strains were found to produce spores that were tested and proved to be effective in the control of locusts and grasshoppers. *Metarhizium* is found every where in the world; therefore, government should encourage researches in order to produce mycopesticide for the control of locusts and grasshoppers.
Keywords: Locusts, grasshoppers, *Metarhizium anisopliae, Beauveriam bassiana, Metarhizium flavoride,* and *Entomophaga grylli*

INTRODUCTION

The fungus *metarhizium anisopliae* was formally known as *Entomopthora anisopliae*. It grows naturally in soils throughout the world and causes disease in various insects by acting as parasite; it thus belongs to the entomopathogenic fungi. It is known to infect over 200 insect species including termites[1]. It is currently being used as a biological insecticide to control a number of pests such as grasshoppers, termites, thrips etc. and its use in the control of malaria transmitting mosquitoes is under investigation [2].

The disease caused by the fungus is called green muscarding disease because of the green colour of its spores. When these mitotic (asexual) spores (called conidia) of the fungus come into contact with the body of an insect host, they germinate and the hyphae that emerge penetrate the cuticle. The fungus then develops inside the body eventually killing the insect after a few days; this lethal affect is aided by the production of insecticidal cyclic peptide (destruxins)[3]. The cuticle of the cadaver often becomes red. If the ambient humidity is high enough, a white mould then grows on the cadaver that soon turns green as spores are produced. Most insect living near the soil have evolved natural defenses against entomopathogenic fungi like *Metarhizium anisopliae:* This fungi is therefore locked in an evolutionary battle to overcome these defenses, which has led to a large number of isolates (or strains) that are adapted to certain groups of insects [4].

Research on *Metarhizium* began in the early 1990s in Africa and preliminary results were so promising. Between 1997 and 2000 field trials covering areas of several hundred hectares showed that *metarhizium* could reduce the numbers of locust hoppers in treated bands by more than 90 percent. It has been reported that the nature of the fungus eventually kills the insect with the greatest mortality occurring some times between 7 to 15 days after treatment (depending upon ambient temperature) . This success led to the first operational use of *Metarhizium* any where in the world during 2000-2001 in which over 20,000 hectare of bandy and swarm of Australian plague locust were aerial treated [5].

Metarhizium spores (the reproduction stage of the fungus) were suspended in oil to form a product termed as "Green Guard". Oil formulation's allow the application of fungi spore under dry conditions, and is compatible with existing ultra low volume (ULV) application techniques for locust control [6]. *Metarhizium anisophae* was named after the insect it was originally isolated from, i.e the *Anisopliaeaustriaca.* It is a mitosporic fungus with asexual reproduction, which was formally classified in the form class hyphomycetes of the form phylum Deuteromycota (also known as fungi imperfecta). *M. anisopliae* does not appear to infect human or other animals and is considered safe as insectide. The microscopic spore are typically sprayed on affected areas. A possible technique for malaria control is to coat mosquito nets or cotton sheet attached to the wall with the spores . Locust and grasshopper often cause extensive and serious damage to crops in many parts of Africa and Asia. Locusts are well known for their potential of invading cropping area in swarms of million of individuals leaving behind devastated fields and plantations. Locust and grasshopper control is currently carried out with chemical pesticides and for many years the product of choice was "dieldrin" and concern about its negative impact on the environment caused it to be prohibited in most countries [6].

This research work was designed to develope a mycopesticide based on the spore of the insect pathogenic fungus *metarhizium anisopliae* var. acridum to serve as a substitute to the chemical pesticide and

reduce drastically the environmental pollution caused by chemical pesticide

MATERIALS AND METHODS

Sample Collection

Ten live insects of the specie *Schistocerca gregaria* (Forsk) were collected from- Tombo field in Gwazange area of Argungu local government in kebbi state, Nigeria. The samples were collected in a clean envelop labeled and transported to the laboratory for analysis.

Temperature simulation

A cage was constructed and the live insects were placed in the cage and the cage was kept in the temperature simulator in which they were fed. Temperature and time was recorded within and outside the simulator for three days under dry condition and was recorded between 28°C - 30°C. Humid condition was maintained by wetting the simulator morning and evening in order to make the fungal attack possible during which the temperature and time were also recorded for seven days and the temperature was recorded between 12^{o} C - 24°C After a week under humid condition some insects were found moving in a jerky passion, some were not feeding and in the second week 3 insects were found dead. Two out of the three insect appeared to show external symptoms characteristic of the fungus *Metarhizium* i.e the abdomen was hard with appearance of striking reddish colour of the cuticle[7-8].

Incubation period

Two insects with external characteristic symptoms of the fungus *Metarhizium* were prepared for incubation. Each sample was placed in a humid chamber and incubated under humid condition at a temperature of 12°C - 24°C for seven days. Dense green cushion of conidia germinated all over the insects cuticle after seven days [8].

Media preparation

Sabouraud dextrose agar (SDA) was prepared according to manufacturer's instructions. 39g of the agar was weighed aseptically on mettles balance (p. 163) and poured into a conical flask. 500ml of distilled water was added, stired and heated to dissolve properly. 0.5g of chloromphenicol was added to inhibit the growth of bacteria; it was then corked with cotton wool and aluminum foil and sterilized in an autoclave at 121°ofor 15 minutes. It was then allowed to cool to a temperature of 40°c and poured into petridishes aseptically. The media was then allowed to solidify in the plates.

Fungal sub-culture

Using a sterile inoculating needle, a small portion of dense cushions of conidia from insect cuticle was picked and inoculated on two plates of the prepared media and incubated at room temperature for seven days.

Identification of fungi

The culture media were first examined macroscopically to determine the colour and types of colonies of each isolate. The visual observation was used to ascertain whether the organism grew fast or slowly.

Microscopic examination

Fungi were identified using a standard method. Using a glass slide, a drop of lactophenol cotton blue was placed on the centre of the glass slide using sterilized inoculating needle. A portion of the fungal growth was gently picked and placed in the drop of lactophenol and teased. It was then covered with cover slip. The slide was viewed under microscope with a low magnification (40x). Pure culture were obtained by sub-culturing from the first plates, this was carried out by picking from the growing edge of fungi with sterilized inoculating needle. Fresh sterile petridishes containing sabouraud"dextrose agar were inoculated. The plates were incubated at room temperature.

Screening of isolates for mycopesticide Production

The two agar plates each inoculated with spore from samples were screened from mycopesticide production using adopted method [9-10].

1. ***Metarhizium spp***

- *Growth of Metarhizium anisopliae* on insect forms a green crust**.**
- Growth of Metarhizium anisopliae on PDA forms colonies with white mycelial margin with clums of conidiophores which become coloured with the development of the spores.2.

2. ***Beauveria spp***

- Growth of *Beauveria bassiana* on insect forms white to pale yellow crust.
- Beauveria bassiana grows radially on PDA and colonies are generally white at the age becoming cream to pale yellow, occasionally reddish.

Characterizationof isolates for mycropesticide production.

The fungal strains on each plate were characterized for spore production. The two plates were incubated at room temperature for seven days. It was observed that *Metarhizium anisopliae* produced significantly more conidia than *Beauveria bassiana* which indicated that *Metarhuium is* more effective.

Preparation of technical material – mycopesticide

Fungi from the stock culture were used to inoculate a liquid culture media based on brewers yeast and sucrose. Fermentation took place under sterile, aerated condition for three days. The resulting "soup" was used to inoculate 1kg of 3 batches of sterile, boiled rice in autoclavable bags in plastic bowls. Conidiation took place after 10 days. The rice bags were opened and left

to dry for 3-7 days before spores were harvested. The spores were sieved off the rice using 150 mesh sieve, the sieve and the collecting bag were closed to prevent the spores escaping. The separated spores were further dried to achieve final moisture contents of less than 6% .

Diluting formulated spores in oil

Metarhizium spore-paste was made using kerosene and appeared light green in colour. The paste was then diluted in groundnut oil for spore counting procedure. The required concentration of conidial suspension is between $IxlO^6$ and $IxlO^7$ conidia/ml.

Haemacytometer preparation

The haemacytometer was dried and free of grease such as finger prints etc. some saliva was wiped over the two edges of the cover slip using a clean finger, it was placed on the haemacytometer and pressed gently until rainbow could be seen (Newton's rings). Pasteur pipette was used to the small amount of the diluted solution and the solution was dropped at the edge of the slip, it was left for few mmutes until the spores were settled.

Counting of spores

25 large squares on haemacytometer grid were used to count the 'spore. Since the concentration was weak, the counting was done between 30 and 300 spores on each haemacytometer grid. Two counts were 'made in order to get accurate estimate of the spore concentration.

Calculating the concentration

25 large squares were counted and the number of spores in each large square was counted, the numbers of spores counted in the squares were added up. The relationship, X = a + b was used to calculate the mean spore count, 2

Where; X = mean count of 25 squares

a + b =• totals for each grid

0.1 microlitre of spore suspension was used over 25 squares.

Then c = number of dilutions,

C = concentration of spores in the original solution,

Then concentration = ex 10".

Bioassay

Three plastic boxes were found and labeled A, B and C which were not air tight and a dried tissue paper was placed in the bottom of each box. One insects was placed in each box, one of the boxes was used as a control. Two microliter spores/ ml of each strain was obtained, the two different measurements were inoculated to samples A and B respectively. The insects were placed back in the boxes and stored at 30°c in 12 hours light and 12 hours dark. The mortality was assessed every day. The two insects were deed after three to seven days and the control alive.

RESULTS

The research work was conducted under tropical climate of Sokoto State in Nigeria. Research of this nature requires a temperate climate i.e. an atmosphere of' low temperature and high humidity since fungi is more active in moist conditions. In order to meet up the growth characteristics of the fungus in question, a temperature simulator was set up and consequently, the desired results were achieved. Temperature ranges were recorded within and outside the temperature simulator under dry and humid conditions for pre-incubation periods respectively for three (3) days each. From the research conducted on entomopathogenic fungi associated with mycopesticide production, the following fungal species were isolated and identified as *metarhizium anisopliae* (metschiiikoff) sorokin, and *Beauveria bassiana* (Balsamo) vuillenim.

Table I: showed the temperature ranges for pre-incubation period under dry condition. In each day, time and temperature were recorded. In day 1, day 2, and day 3 the time at which the temperatures were recorded varied from 9:50am to 5:30pm and temperatures varied from *28P(^ to* 30°C for both within the temperature" simulator and atmospheric temperature. It could be deduced from Table I that temperature could appreciate within the temperature simulator beyond 29°C between l 7:00 pm and 12:00 midnight; which would not be suitable for the desired incubation procedures**.**

Table 2 showed the temperature ranges for pre-incubation period under humid conditions. In each day, time and temperature were recorded. In day 1, day 2**,** and day 3 the time at which the temperatures were recorded varied from 8:00am to 9:25pm and temperatures varied from 20°C to 25°C within the temperature simulator and 20°C to 27°C atmospheric temperature. It could be deduced from table II * that temperature within the temperature simulator could depreciate between 8:00pm and 4:00am which would be suitable for the desired incubation procedures.

Table 3 indicated the temperature ranges for incubation period under humid condition. In each day, time and temperature were recorded. In day 1, day 2, day 3, to day 7 the time at which the temperatures were recorded varied from 8:00am to 7:15pm and temperatures varied from 14°C to 24°C within the temperature simulator and 12°C to 25°C atmospheric temperature. It could be deduced from table III that the temperatures within the temperature simulator could depreciate from 8:00pm to 4:00am and both the temperature within and outside the simulator would be suitable for the desired incubation procedures. After having set up the temperature simulator conducive for the conduct of the research, the infected insects were incubated in humid chambers under humid conditions.

Table I: Temperature ranges for pre-incubation period under dry condition

Days	Time	Temperature Simulation	Atmospheric temperature	Remarks
Day 1	10:35am	280C	'28°C	Not Suitable For
	12:35pm	29°C	29"C	incubation
	2:53pm	29°C	30°C	
Day 2	10:00am	29°C	29°C	
	2:30pm	29°C	30°C	
	4:50pm	28°C	29°C	
Day 3	9:50am	29°C	29°C	
	ll:30pm	30°C	30°C	
	5:30pm	29°C	29°C	

Table 2: Temperature ranges for pre-incubation period under humid condition

Days	Time	Temperature Simulation	Atmospheric temperature	Remarks
Day 1	10:50am	220C	26°C	Not Suitable For
	8.00pm	26°C	27"C	incubation
	9.25pm	20°C	20°C	
Day 2	11:am 4:50pm	22°C	24°C	
	8:30pm	26°C	29°C	
		22°C	24°C	
Day 3	10:00am	26°C	25°C	
	2:30pm	22°C	27°C	
	4:50pm	22°C	20°C	

Table 3: Temperature ranges for incubation period under humid condition

Days	Time	Temperature Simulation	Atmospheric temperature	Remarks
Day 1	8:00am	14°C	130C	Suitable
	1 l:55am	1°C	23°C	for incubation
	3:55pm	20°C	250C	
Day 2	7:40am	13°C	120C	
	12:12pm	20°C	25°C	
	4:30pm	22°C	24°C	
Day 3	7:32am	14°C	12°C	
	5:30pm	24°C	24"C	
Day 5	9:15am	20°C	250C	
	2:37pm	20°C	240C	
	5: llpm	19°C	20"C	
Day 6	7:15am	150C	12°C	
	ll:00am	18"C	210C	
	4:50pm	170C	190C	
Day 7	7:15am	17"C	16°C	
	12:30pm	20"C	24°C	
	4:50pm	15°C	180C	

DISCUSSION

Fungi are among the largest species of organisms studied in microbiology, often no special staining technique or biochemical studies are necessary to identify the genus side. Their structural differences are often readily visible microscopically.

Locusts and grasshoppers after cause extensive and serious damage to crops iri many parts of Africa and Asia. Locusts are well known for there potential of invading cropping areas in swarms of millions of individual leaving behind devastated fields and plantations. Luckily, the invasions are infrequent and may be followed by long periods of recession. In contrast, grasshoppers form a more chronic problem causing serious yield losses in most years. Following this, biological control of locust and grasshoppers project started late in 1989 with the objective of developing a biological means of controlling locusts and grasshoppers. It became apparent that oil formulations of deutromycetes fungal spores offered the most promising option. Such fungi will grow on artificial substrates and so can be mass produced quickly in large quantities. Their spores are lipophilic, they suspend more readily in oils than in water. Fungi in nature are most active under moist conditions, part of the original concept was the formulating spore in oil might overcome this constraining requirement for high humidity.

Prior and Great head [11] were reported to have isolated *Metarhizium* and *Beauveria* species particularly *Metarhizium anisopliae* and *Beauveria bassiana* respectively from a species of grasshopper known as *Schistocerca gregaria* (Forsk) after having infected by these strains of fungi. The insects were incubated in a humid chambers at a temperature of20°C-28°C. The *Metarhizium anisophia* formed a green crust like velvet which was found on both inner and outer walls of the insect cuticle, when cultured on agar, *Metarhizium anisopliae* grew radially on PDA and MEA. The colonies on PDA showed a white mycelial margin with clumps of conidiophores which became coloured with the development of the spores. The *Beauuaria bassiana* on the other hand, showed white to pale yellow crust on insect and when cultured on PDA grew radially with white colonies and the edge became cream to pale yellow.

Magalheas et al[12] ; isolated strains of *Metarhizium* as *Metarhizium anisopliae* and *Beauueria bassiana* respectively from *Schistocerca gregaria.* The two isolates CG 423 (M. *anisopliae var.* acridum) and CG 425 *(B. bassiana}* were cultured in SDA after retrieval from storage in liquid nitrogen. For the bioassay, the fungi were cultured in rice with brewers yeast and sucrose. The insects were individually inoculated with 3pl of a conidial suspension (5000 conidia/insect) applied on the right pleural region with a micropipette. The insects were maintained in 17cm x 21cm x 25cm cages at 25°C. Both fungi were equally virulent against *Schistocerca gregaria,* but M. *anisopliae* produced more conidia internally than *B. bassiana* at low humidity. Externally, there was no sporulation when infected insects were incubated at 75% RH and M *anisopliae* var. acridum produced significantly more conidia than *B. bassiana* at 100% RH.

Although the two researches conducted by Prior and Greathead and that of Magalhaes *et al;* were carried out in temperate climates, this research conducted in the tropical climate yielded results that are in conformity with the results obtained by Prior and Greathead and that of Magalhaes *et ai*, through the help of temperature simulation[12].

CONCLUSION

Sequel to the international community's concerned about the development of alternative control methods for invading locusts and grasshoppers, a mycopesticide based on the spores of insect pathogenic fungus, *metarhizium anisopliae* var. acridum was produced. This fungus, which appeared to be specific to species of short-horned grasshoppers acridoidea and pyrgomorphoidea), is widely distributed in Africa and under favourable conditions, can cause local epidemic in grasshopper or locust populations. Its biological and physical properties make this fungus an ideal candidate for augmentative biological control. Spores of M. *ansopliae* var. acridum can be easily, mass-produced.

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