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Isolation and identification of entomopatogenic fungi from unlittered soil in Bauchi metropolis used as pest control.

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Abstract
Entomopatogenic fungi were isolated and characterized using insect as bait collected from unlittered soil in Abubakar Tafawa Balewa University Bauchi at three different locations. *Metarhizium anisopliae* was isolated from 5 of 9 (55.5%) holes (soil samples) baited. The isolate was obtained from all the locations sampled except soil from the onion garden, and it was more frequently obtained from soils under pepper cultivation compared to soils with natural vegetation. The pathogenicity of *Metarhizuim anisopliae* isolate was determined using spray and immersion method. The isolate extracted by immersion method was more effective in the control of agricultural pest. The result also showed that entomopathogenic fungi isolated locally, could be used for integration of control for various agricultural pests.

Key words: Agricultural pests, integration, isolate, pathogenicity, soil samples

Introduction

Entomopathogenic fungi are fungi which attacks insect pest that are of economic importance to agriculture and disable or kill them. They are distributed in a wide range of habitats including aquatic forest, agriculture, pasture, desert and urban habitats (Lacey *et al.*, 1996). Their ability to regulate insect populations has been studied in tropical and temperate habitats (Meyling and Eilenberg, 2007).Soil is considered an excellent environment shelter for entomopathogenic fungi since it is protected from Ultra Violet radiation and other adverse abiotic and biotic influences (Keller and Zimmerman, 1989). They are environmentally safe and are very virulence caused by contact and action through penetration of the fungi into the insect. Entomopathogenic fungi have a global distribution (Zimmermann, 1993) and wide insect host ranges. Bio-pesticides based on entomopathogenic fungi show promise for insect pest management.

A large amount of genetic diversity has been reported in *Metarhizium anisopliae* and *Beauveria bassiana* (Bidochka *et al*., 1994), and the potential existence of strains adapted to various hosts, environmental conditions, conidial survival and competitive saprophytic ability can profoundly influence their virulence. Studies have shown that strains can be distinguished by their different levels of proteases, chitinases and lipases (Varela and Morales, 1996).

The anamorphic entomopathogenic fungi *Beauveria bassiana and Metarihizum anisopliae* are natural enemies of a wide range of insect *and Hirsutellar hossilisnsis* a natural enemy of nematode or mite (Roberts and St. Leger, 2004). Pest biological control is being considered as an important part of integrated pest Management (IPM), which is a more ecological friendly

strategy than conventional chemical pest control. For example, using microorganisms such as *Beauveria bassiana, Metarihizum anisopliae and Verticillium lecanii* can be used in greenhouses because of its capacity to infect aphids and whiteflies.

The rapid acceleration of the of chemical pesticides in agricultural production has in many cases lead to increased production, but it has also had several adverse effects; deteriorating the environment in different ways such as contaminating water source and bottom sediments. Pest have developed resistance against many pesticides and the pesticides can impact non- target organism negatively such as animals and humans (Amuwitagama, 2004).The Swedish National Food Administration revealed that one third of the samples from cereals, fruits and vegetables contained traceable amounts of at least two pesticide. Pesticides have many disadvantages such as harming non – target organism (Messmer and Dahl, 2009). However the alternative to pesticides is biological control. There are four strategies: – classical biological control, inoculation biological control, inundation biological control and conservation biological control.

In this study, the strategy to be used is inoculation biological control. These emphasize on the use of living organism to suppress the population density of a specific pest control organism, making it less abundant or less damaging than it would otherwise been (Eilenberg and Hokkanen, 2006).Biodiversity as represented by naturally occurring enemies of pest such as Bacteria, fungi, insect and viruses is an important element in biological control system for pest control management.

The contribution of the entomopathogenic component of this biodiversity to the regulation of pest populations has often been ignored (Gurr *et al*., 2003) and when it has been acknowledged, it has usually been discussed if the introduction of exotic strains of fungi, or the augmentation of endemic strains, is an appropriate bio-control strategy (Carruthers and Onsager, 1993).

The entomopathogenic fungi are considered natural mortality agents and environmentally safe. Thus, the present study of entomopathogenic fungi as an important microbial control agent that can have a long term use and be an alternative to the use of chemical pesticides. The objectives of the study are twofold: to isolate and identify, entomopathogenic fungi from different soil locations in Abubakar Tafawa Balewa University, Bauchi, and to evaluate the potency of the fungal isolates against some insect pest.

Materials and Methods

Sample Collection

Insects (Rain beetle) were collected from a field in the college of Agriculture Bauchi and a total of eighteen of the insects were soil baited. The insects were buried at three unlittered soil locations at Abubakar Tafawa Balewa University Bauchi.

Namely;

1. The Onions garden located at River Sambo.
2. Pepper (*Capsicum anuum L.*) garden, located at the University farm.
3. Botanical Garden located at the front of the biological sciences laboratory.

Soil Baiting

At each of the three locations, three (3) holes were randomly dug with a minimum distance of 15m between them, depth of 5cm and 5cm diameter using farming hoes. To each of the holes were added 30ml of non-chlorinated, purified water. The insect samples were left for 30 minutes to let the soil absorb the water. After 30 minutes the insects were buried, two (2) in each of the three (3) holes.

Isolation of the Entomopathogenic Fungi

The isolation of the entomopathogenic fungi was achieved through soil bating. The insects were harvested after 7 days, in petri dish with a forcep. The dead insect with or without incipient, visible external fungal growth were washed with 70% ethanol and allowed to dry under sterile condition for 5 minutes. Then, the dead insect was scrapped with sterile wire loop under laminar flow hood to an already prepared Potatoes Dextrose Agar plates and it was

incubated for up to7days at room temperature of 25^0C.
After incubation, fungal growth (spores) growing on the Potatoes Dextrose Agar plates grew with their distinctive colours.

Identification of Entomopatogenic Fungi
The Entomopatogenic Fungi isolates identified were identified macroscopically using Entomopatogenic Fungi atlas and microscopically, from cultures to observe the following characteristics (Humber, 1997).

Potency Test
The potency test was carried out to determine the virulence of the isolate on various Agricultural pest. Three different Agricultural pests used were: Sting Bug (*Halymorpha halys*), Lady beetle (*Epilachninae varivestis*) and Bean leaf beetle (*Cerotoma trifurcata*). Two of the agricultural pest was used, for the isolate and two agricultural pests for the control making a total of 18 Agricultural pests. The Agricultural pest was treated, using the spraying method and immersion method.

Spraying method
Spores were collected from the pure isolate using a sterile wire loop at a concentration of $1x10^7$(measured using heamocytometer). Two test insects were placed each in a petri dish, and a control group was set. The insect were sprayed with the conidia on the insects and covered (Lacey, 1997). The growth was examined daily and the time and date were recorded. The insects died after three days, and control was still alive.

Immersion method
Spores from the solid media were collected and mixed into 5ml of distilled water to a concentration of $1x10^7$. The suspension was shaken vigorously, and the various live insects were immersed into it and placed in a petri dish having filter paper for a humid environment. The growth was examined daily as the time and date were recorded. The insects died after two days and the control was still alive.

The agricultural pests infected with the isolate show resultant growth. They were identified by a dehydrated, shrivelled aspect with sparse mycelium colonization and abundant colonization (Eyal *et al.*, 1994).

Control test
Two each of the three different pests were placed in the petri dish (control for each method) and it was kept in the same condition as the treated pest.

Results
A total of eighteen insects were buried on three holes (2 for each hole), at the three locations (Onion garden, Botanical garden, Pepper farm). *Metarhizium anisopliae* was isolated from 4 insects, in botanical garden and 2 insect yielded no growth, growth also occurred on the 6 insect buried on pepper garden. No growths were isolated on the 6 insects buried in the Onion garden.

Table 1: Isolate obtained from different locations.

Locations	Hole numbers	*Metarhizium anisopliae*
Onion Garden(River Sambo)	1	--
	2	--
	3	--
Botanical garden	1	++
	2	++
	3	--
Pepper Farm (University farm)	1	++
	2	++
	3	++

Key: **-** = number of insect without growth,
+ = number of insect with growth.

Table 2 gives the effects of isolate on the various adult Pests. The Potency test was carried out on 12 insect pests using spraying method. Two each of the three different insect pests (Sting bug, Lady beetle and Bean leaf beetle) were used as treatment, and two each for the three different insect pests were used to set the control group. They were then incubated at room temperature, after three days of incubation, one each of the Sting bug and Bean leaf beetle died on the second day and two of the lady beetle was still alive. On the third day all the treated pest died, while the controls were still alive.

Table 2: Potency test of isolate using spray method on the Agricultural pest.

S/N	Name of insects	Day 1 (No. alive)	Day 2 (No. alive)	Day 3 (No. alive)	Control (No. alive)
1	Sting Bug	2	1	0	2
2	Lady Beetle	2	2	0	2
3	Bean leaf Beetle	2	1	0	2

Table 3: Potency test of isolate on the Agricultural pests by immersion method.

S/N	Name of insects	Day 1 (No. alive)	Day 2 (No. alive)	Control (No. alive)
1	Sting Bug	2	0	2
2	Lady Beetle	2	0	2
3	Bean leaf Beetle	2	0	2

Table 3 give the effects of isolate on the various adult Pests. The Potency test was carried out on 12 insect pests, using immersion method. Two each of the three different insect pests (Sting bug, Lady beetle and Bean leaf beetle) were used as treatment, and two each for the three different insect pests were used to set the control group. They were then incubated at room temperature and all the treated pests died on the second day, while controls were still alive.

Discussion

The aim of this study was to isolate entomopathgenic fungi from our environment which could be a biological control agent and to test for its potency on Agricultural pest. *Metarhizuim anisopliae* was the isolate obtained from two of the three locations soil baited. The recovery of *Metarhizuim anisopliae* was because it's ubiquitous in soil. Similar findings have been reported by (Diver, 2000) and it not influenced by humidity of the soil samples. Laboratory trial as demonstrated the possibility of infecting insects with fungal entomopathogens at relatively low ambient humidities (45-70%) (Fragues *et al.*, 1997; James *et al.*, 1998; Arthurs and Thomas, 2001). However, the level of humidity influences the transmission of insect pathogens. High environmental moisture has been found to be important for fungal entomopathogens to sporulate (that is to produce infective asexual conidia on the host cadavers during the necrophytic phase of the fungus). (Hajek and St. Leger, 1994).

The isolate *Metarhizuim anisopliae* was not found on onion garden because of the continuous irrigation process on the farm; *Metarhizuim anisopliae* preferred un-irrigated and cultivated soils as compared to uncultivated soil. Similar findings have been reported by Rath *et al.*, 1992; Vanninen, 1996 and Bidochka *et al.*, 1998). Several factors influence the survival of fungal entomopathogens in soils (Fargues and Robert, 1985; Daoust and Pereira, 1986), and the general finding is for

Metarhizuim anisopliae to persist for extended periods in soils.

The rate of mortality when pest were treated with conidial suspension by immersion was high compared to when sprayed with the conidia. The fungal isolates showed a high level of mycosis ranging from 85-97% (compared to 0% in the control group) meaning that majority of the insect died of the fungal infection. Other studies support that *Metarhizuim anisopliae* have a high lethal effect on other insect used (Makaka and Chen, 2005; Kannan *et al.*, 2008).

The fungal isolate can withstand adverse conditions as resting spores that enables it to survive through periods when host are not present (Lacey 1997; Butt, 2001). Once applied, it is present and can be activated when pest attacks the crop and act in a preventive way. In the use of the fungi as Biological Control Agent, knowledge about the insect's behaviour can be useful in order to enhance efficiency and to increase spread of the fungi. In general, entomopatogenic fungi are also cheap to produce in big quantities and easy to store (Al-Degari, 2008). There is a potential increased use of entomopathogenic fungi as Biological Control Agents in the future and it is therefore important to investigate its optimal use on different pest and to optimize the spore concentration for different purposes.

Conclusion

There was no growth in the onion garden because of continuous farming and irrigation on the farm which led to leaching and washing away of top soil as entomopathogens are found 5cm-7cm on the soil surface. The fungal spores were not isolated from a soil rich in humus. The fungal spores were seen on the insect in a more humid environment than those in less humid areas. Entomopathogenic fungi present locally, could be used for integration of control for various Agricultural pests.

Recommendation

The use of entomopathogenic fungi, as an alternative to chemical pesticide or as part of integrated pest management programmes. Farmers should be trained on the use of microbial insecticides for biological control of insect pests as the fungal agents are among the most promising group of biological control agents against insect pests as they infect their host through the cuticle.

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