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Full Length Research Paper

## Sources and abundance of fungi with entomopathogenic potential for control of the cowpea pod borer, *Maruca vitrata* Fab. in Ibadan, Nigeria

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The potential sources and abundance of naturally occurring entomopathogenic fungi with bio-control potential against the cowpea pod borer, *Maruca vitrata*, were investigated by adapting the *Galleria* bait method. Soil samples from five sites: Cow-stead, Piggery and Poultry sites as well as Crops Research Garden (CRG) and Practical Year Training Programme (PYTP) farm for arable crops of the University of Ibadan were used in the study. Soil samples from the different sites and 2<sup>nd</sup> instar larvae that were exposed to the samples of the different soils were assessed for occurrence and abundance of the fungi following standard procedures. Results show nine fungi species from soil samples and seven fungi species to be associated with dead larvae of *M. vitrata*. The most abundant fungi in the soil and dead larvae were *Rhizopus* sp. and *Fusarium* sp. while the most abundant fungus with known entomopathogenic potential was *Beauveria bassiana* followed by *Trichoderma* and *Penicillium* spp. The best sources for collection of the entomopathogenic fungi were the arable crop farms of the PYTP and the CRG sites where active farming activities carried out.

**Key words:** Entomopathogenic fungi, *Beauveria bassiana*, *Trichoderma* and *Maruca vitrata*.

### INTRODUCTION

The pod borer, *Maruca vitrata* is a major field pest of Cowpea, *Vigna unguiculata* (L.) Walp., causing severe yield losses in Nigeria. The challenges posed by this and other field insect pests have constrained many cowpea

farmers to apply synthetic pesticides in order to obtain good yield (Abate and Ampofo, 1996; Atachi, 1998; Adipala et al., 2000; Adu-Dapaah et al., 2005; Adati et al., 2007). However, the use of synthetic pesticides is being

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discouraged due to threat to human, livestock and environmental health (Ton et al., 2000; Thundiyil et al., 2008; Thiam and Touni, 2009).

There is currently a growing concern among farmers and other stakeholders to search for and develop environmentally friendly pest management options that would be sustainable and capable of minimizing pre-harvest losses and enhance production. The use of biological agents especially fungal entomopathogens such as *Beauveria bassiana*; *Lecanicillium lecanii*, *Paecilomyces farinosus* and *Paecilomyces varioti* (Gottwald and Tedders, 1984; Hallsworth and Magan, 1999; Vega et al., 2008); via well coordinated pest management programme has proved to be effective and environmentally safe in managing some pests of crops (Balogun and Fagade, 2004). Among these, *B. bassiana* is reputed to be one of the most widely used entomopathogens for control of many insect pest of crops such as stem borers, beetles, aphids, mites, termites, white flies, mealy bugs and thrips especially via exogenous application as spray formulations (Feng et al., 1994; Shah and Pell, 2003; Tefera and Vidal, 2009).

Aside their comparable effectiveness, the various risk factors associated with the use of chemical insecticides such as development of resistance, pest resurgence, residues accumulation in food chain, environmental and human health risks and high costs have driven scientist and farmers to intensify the quest for alternative strategies via using entomopathogenic organisms for pest management. This has necessitated the need to search for local biotic agents with potential for control of destructive crop pests. The objective of this study therefore is to bioprospect for fungi with entomopathogenic potential via isolation and identification of pathogenic species, their abundance and sources in the local community where local isolates and strains could be readily obtained for research and possible adoption for pest management.

## MATERIALS AND METHODS

### Study site

Investigations were conducted in the Entomology and Pathology Research laboratories of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, at ambient conditions of  $65 \pm 5\%$  relative humidity and temperature of  $27 \pm 3^\circ\text{C}$ .

### Sources of larvae and culture media

The second instar larvae of *M. vitrata* as well as the artificial diet and fresh cowpea pods used in the study were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan. The fresh pods used were plucked from the susceptible cowpea variety - tvs3236. The artificial diet was composed from cowpea flower variety - tvs3236; wheat germ flour, sugar, salt mix, ascorbic acid,

potato dextrose agar (PDA) and stock solution. The stock solution consisted of acetic acid, formaldehyde, vitamin suspensions, choline chloride and potassium hydroxide (Aderanti, 2013; Personal comm. IITA Ibadan, Nigeria).

### Soil sample collection

Potentially, fungi infected soil samples (200 g) were purposefully taken from five different sites with different history of use in the University of Ibadan namely: (A) Piggery Unit of the Teaching and Research Farm (TRF); (B), Poultry Unit of the TRF; (C), the Cow stead site of the TRF; (D), the Crop Research Garden (CRG) of the Department of Crop Protection and Environmental Biology (CPEB) and (E) the Practical Year Training Programme (PYTP) farm site. All were evaluated in four replicates for abundance and diversity of naturally occurring fungi with entomopathogenic potential following standard procedures.

### Isolation of fungi from soil samples

Suspension of soil samples collected from each site was prepared by addition of 1 g soil into 9 ml of sterile distilled water and admixing thoroughly. Thereafter, Serial dilutions ( $10^{-1}$  to  $10^{-5}$ ) of the prepared soil suspensions were made. One millilitre each of the three ( $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$ ) dilutions was poured into sterile Petri dish which was mixed with cooled Potato dextrose agar (PDA) supplemented with lactic acid to avoid bacterial growth and sterilized for 20 min at  $121^\circ\text{C}$ . Four replications were used for each dilution level. The plates were sealed with parafilm before incubation at  $25^\circ\text{C}$  for 7 days. Fungi species isolated were identified and pure cultures were obtained by a subsequent re-isolation by adapting the method used by Mohammadbeigi and Port (2013).

### Isolation of fungi from infected larvae of *Maruca vitrata*

A 200 g sample of each soil sample collected from the various sites already described was weighed and replicated four times. The samples were re-moisturized to 60% water holding capacity with distilled water before fresh cowpea pods of the susceptible variety TVS-3236 were placed on them. Adapting the galleria bait method described by Zimmerman (1986), five 2<sup>nd</sup> instar larvae of *M. vitrata* were introduced into each of the soil samples using a camel hair brush. The larvae were left to feed on the fresh cowpea pods placed on the different soil substrates and examined daily till they died. The dead larvae were retrieved; surface sterilized with 1% sodium hypochlorite and rinsed in three washings of sterile distilled water at the Pathology Laboratory, Department of CPEB. Thereafter, the larvae were placed initially on sterile whatman No 1 filter paper before being plated on PDA which had been sterilized for 20 min at  $121^\circ\text{C}$  and supplemented with lactic acid to prevent bacterial growth. The plates were sealed with parafilm. Fungal pathogens isolated were identified and pure cultures were obtained as already described.

### Data analysis

The experimental design for all trials was completely randomized. Data on number of cfu/ml of samples were analyzed using the analysis of variance (ANOVA) and the mean values were compared by the Least Significant Difference test ( $P \leq 0.05$ ) using SAS statistical software.

**Table 1.** Occurrence of fungi in samples of soil and dead larvae of *Maruca vitrata* raised on different soil samples in Ibadan.

Fungi	Order	Family	Mean number of cfu/ml	
			Soil	Dead larvae
<i>Aspergillus niger</i>	Eurotiales	Trichocomaceae	5.82±2.60	6.82±3.88
<i>A. flavus</i>	Eurotiales	Trichocomaceae	5.55±2.21	5.39±2.22
<i>A. terreus</i>	Eurotiales	Trichocomaceae	4.02±1.75	5.82±2.59
<i>A. ochraceus</i>	Eurotiales	Trichocomaceae	3.05±1.08	5.51±2.78
<i>Rhizopus</i> sp.	Mucorales	Mucoraceae	9.03±3.63	4.59±1.41
<i>Beauveria bassiana</i>	Hypocreales	Clavicipitaceae	5.02±2.20	0.00±0.00
<i>Trichoderma</i> sp.	Hypocreales	Hypocreaceae	4.39±1.17	2.87±0.94
<i>Penicillium</i> sp.	Eurotiales	Trichocomaceae	4.42±1.84	3.02±1.11
<i>Fusarium</i> sp.	Hypocreales	Nectriaceae	6.28±2.32	0.00±0.00
LSD <sub>(0.05)</sub>			10.93	3.16
CV%			58.9%	43.7%

cfu = Colony forming units.

## RESULTS

### Occurrence and abundance of fungi associated with dead larvae of *Maruca vitrata* and soil samples in the University of Ibadan, Nigeria

The occurrence and abundance of fungi associated with each soil sample and the dead larvae of *M. vitrata* from each of the soil samples varied significantly as presented in Table 1. A total of nine species were encountered on both soil and insect larvae exposed to the soil tested. All the nine species were detected in the soil samples whereas only seven fungi species were detected in the dead larvae from each soil sample. In addition, the nine species detected in the soil were from five families and three orders (Table 1) while all the fungal species except *B. bassiana* and *Fusarium* sp. were detected on the dead larvae. The most abundant fungus in the soil was *Rhizopus* sp. (9.03 cfu/ml) and was significantly higher ( $P < 0.05$ ) than *Fusarium* sp. (6.28 cfu/ml) > *Aspergillus niger* (5.82 cfu/ml) > *A. flavus* (5.55 cfu/ml) > *B. bassiana* (5.02 cfu/ml) > *Penicillium* sp. (cfu/ml) > *Trichoderma* sp. (4.39 cfu/ml) > *A. terreus* (4.02 cfu/ml) > *A. ochraceus* (3.05 cfu/ml). In the dead larvae however, the seven species found were from four orders and three families (Table 1). The most abundant species detected on the dead larvae was *A. niger* (6.82 cfu/ml) > *A. terreus* (5.82 cfu/ml) > *A. flavus* (5.39 cfu/ml) > *A. ochraceus* (5.51 cfu/ml) > *Rhizopus* sp. (4.59 cfu/ml) > *Penicillium* sp. (3.02 cfu/ml) while the least was *Trichoderma* sp. (2.87 cfu/ml). The coefficient of variation for soil was 58.9% while for the dead larvae it was 43.7%, indicating that fungal pathogens were better dispersed on the insect larvae than in the soil samples.

### Influence of sources of soil samples on the occurrence and abundance of fungi in soil samples from selected sites in the University of Ibadan

From the total of nine fungi isolated and identified in the soil substrates from the different sites (Table 1); only the soil samples from the PYTP and the Poultry site had the full complement of all the nine fungi. Eight were detected in each of the soils from Piggery, Cowstead and the CRG sites (Table 2). The most abundant fungus in soil from the PYTP site was *Rhizopus* sp. (10.58 cfu/ml), followed by *A. niger* (9.76 cfu/ml), while the most abundant fungus at the Poultry site was *Aspergillus flavus* (9.57 cfu/ml) followed by *Rhizopus* sp. (8.20 cfu/ml). Similarly, the most abundant fungus in the Cowstead soil sample was *Rhizopus* sp. (9.05 cfu/ml) followed by *A. niger* (7.98 cfu/ml). *Rhizopus* sp. (9.67 cfu/ml) and *Trichoderma* sp. (6.88 cfu/ml) were the most abundant fungi in soil samples from the Piggery and CRG sites respectively. Apart from *A. ochraceus* and *Trichoderma* sp. with significantly higher number of colony forming units from the PYTP soil sample; the difference between the number of colony forming units of *A. ochraceus* and *Trichoderma* sp. from all the samples were not significant. The differences in the number of colony forming units in *A. flavus*, *A. terreus* and *Rhizopus* sp. were also not significant ( $P > 0.05$ ) on the soil samples from the piggery site but these were significantly higher compared to other fungi species. Similarly, the differences in the number of colony forming units of the different fungi in the soil sample from the poultry site were not significantly different ( $P > 0.05$ ) except for *Trichoderma* sp. and *Penicillium* sp. Similarly, differences

**Table 2.** Occurrence and abundance of fungi in soil samples from selected sites in Ibadan

Pathogen	Mean cfu/ml					LSD <sub>(0.05)</sub>
	PYTP	Cow stead	Piggery	Poultry	Crop garden	
<i>Aspergillus niger</i>	9.76±3.65*	7.98±1.25	3.20±1.47	4.65±1.10	3.53±0.95	4.14
<i>A. flavus</i>	2.53±1.14	5.66±2.50	5.22±1.55	9.57±3.65	4.77±1.35	2.32
<i>A. terreus</i>	4.52±1.13	0.00±0.00	5.88±2.41	3.14±1.08	4.22±2.37	3.15
<i>A. ochraceus</i>	6.98±2.95	3.43±2.16	0.00±0.00	1.20±1.05	3.65±0.70	4.85
<i>Rhizopus</i> sp.	10.58±3.42	9.05±2.25	9.67±2.78	8.20±3.89	0.00±0.00	6.15
<i>Beauveria bassiana</i>	7.65±2.30	3.56±1.75	4.20±1.70	3.14±1.80	6.53±3.42	3.25
<i>Trichoderma</i> sp.	5.42±1.67	4.63±1.89	3.30±1.42	1.72±0.70	6.88±4.22	1.58
<i>Fusarium</i> sp.	3.18±0.85	6.05±3.00	5.01±1.64	4.29±1.96	3.56±1.56	2.42
<i>Penicillium</i> sp.	6.38±2.34	5.64±2.50	7.82±3.05	5.71±1.22	5.86±1.29	5.14
LSD <sub>(0.05)</sub>	4.75	2.55	2.44	3.19	2.97	

cfu = Colony forming units.

**Table 3.** Occurrence of fungi on dead larvae raised on cowpea pods on soils from different sites in Ibadan.

Pathogen	Mean abundance (cfu/ml)					LSD <sub>(0.05)</sub>
	PYTP	Cow stead	Piggery	Poultry	Crop garden	
<i>Aspergillus niger</i>	5.05±2.10	13.7±5.20	4.36±1.39	5.65±2.20	5.33±2.60	3.92
<i>A. flavus</i>	7.75±4.50	4.43±2.50	7.78±3.50	3.88±1.16	3.10±2.80	2.16
<i>A. terreus</i>	5.23±2.65	9.33±3.75	7.55±3.65	2.72±1.40	4.53±2.80	3.61
<i>A. ochraceus</i>	2.56±3.10	9.43±3.80	3.96±1.10	7.25±3.69	4.35±2.48	2.83
<i>Rhizopus</i> sp.	3.65±0.75	2.70±1.10	5.67±2.95	4.87±1.90	6.08±3.79	4.86
<i>Trichoderma</i> sp.	2.16±1.94	3.19±2.35	4.21±1.56	1.80±0.75	2.98±0.97	0.87
<i>Penicillium</i> sp.	4.00±1.95	3.10±1.20	4.21±2.14	1.80±1.05	2.01±1.15	0.39
LSD <sub>(0.05)</sub>	4.22	4.51	2.65	3.01	3.27	

cfu = Colony forming units.

between the numbers of colony forming units of all the fungi detected from the CRG samples were not significant except for *A. niger*, *A. ochraceus* and *Fusarium* sp. However, the concentration of *A. niger* from the PYTP and the Cowstead was significantly higher than those found on other substrates (Table 2). The number of the colony forming units of *B. bassiana* and *Trichoderma* sp. found was significantly higher on the PTYP soil sample (7.65 cfu/ml) followed by the Crop Garden (6.53 cfu/ml) than on all other samples.

#### Occurrence of fungi on dead larvae of *Maruca vitrata* raised on cowpea pods placed on soils from different sites in the University of Ibadan

A total of seven fungi: *Rhizopus* sp., *A. terreus*, *A. niger*, *Trichoderma* sp., *A. ochraceus*, *Penicillium* sp. and *A. flavus* were detected on all the samples (Table 3). *A.*

*flavus* was the most abundant on the larvae from pods on the PYTP site soil sample while *A. niger* was the most abundant on the larvae from pods from the Cowstead site (13.7 cfu/ml). Similarly, the most abundant fungi on the larvae from pods from the Poultry site (7.25 cfu/ml) was *A. ochraceus* while *A. flavus* was the most abundant on the larvae raised on the soil from the Piggery site (7.78 cfu/ml). *Rhizopus* sp. was the most abundant on larvae from soil samples from the CRG. From this study, the sites from which the soil samples were taken did not significantly influence the number of colony forming units of the detected fungi. For example, the number of cfu of the different fungi detected on the larvae from the pods raised on PYTP soil was not significantly different from those from the other sites except for *A. flavus*, *A. ochraceus* and *Trichoderma* sp. Yet, the number of CFUs of *A. niger* on the pods from Cowstead site varied significantly, although only *A. flavus* and *A. terreus* had significant higher number of the cfu compared to other

**Table 4.** Abundance of fungi species at different dilution levels.

Pathogen	Mean abundance (cfu/ml) / dilution level			
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	LSD <sub>(0.05)</sub>
<i>Aspergillus niger</i>	3.60±1.25	3.50±2.16	2.30±0.97	2.52
<i>A. flavus</i>	1.05±0.79	1.20±0.63	0.00±0.00	2.20
<i>A. terreus</i>	4.70±1.04	1.35±0.81	0.85±1.80	3.80
<i>A. ochraceus</i>	2.05±0.98	2.20±1.70	0.30±0.76	2.30
<i>Rhizopus</i> sp.	6.15±1.04	4.90±0.81	3.65±1.81	2.56
<i>Beauveria</i> sp.	1.65±0.54	1.55±0.39	0.90±0.87	1.45
<i>Trichoderma</i> sp.	2.25±0.65	2.25±1.12	1.00±0.51	2.78
<i>Penicillium</i> sp.	1.20±0.45	0.70±0.35	0.50±0.78	1.30
<i>Fusarium</i> sp.	1.35±0.29	0.40±0.22	0.35±0.49	1.80
LSD <sub>(0.05)</sub>	2.17	2.79	4.85	

**Table 5.** Abundance of *Trichoderma* sp. in samples from different locations at different dilution levels.

Parameter	Mean Abundance / dilution level (cfu/ml) (n=4)			
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	LSD <sub>(0.05)</sub>
PYTP	6.50±1.20	4.50±1.10	1.25±0.95	4.08
Cow stead	1.25±0.25	0.95±0.00	0.00±0.00	0.42
Piggery	0.00±0.00	1.50±0.20	0.25±0.50	0.65
Poultry	0.75±0.36	0.75±0.25	1.25±1.05	0.92
Crop garden	4.00±2.10	4.75±2.75	2.50±1.65	3.75
LSD <sub>(0.05)</sub>	2.25	2.25	1.00	

fungi detected on the larvae from the Piggery site (Table 3).

#### Effect of serial dilution on the abundance of fungal pathogens from soil substrate and dead larvae of *Maruca vitrata* in University of Ibadan

The number of cfu/ml of fungi detected in soils from different sites in Ibadan reduced significantly with increase in the dilution levels of the samples except for *A. flavus* and *A. ochraceus* (Table 4). The abundance which was determined by the number of colony forming units (cfu) of each of the detected fungi was highest at 10<sup>-3</sup> followed by 10<sup>-4</sup> and 10<sup>-5</sup>. At the dilution level 10<sup>-3</sup>, the most abundant fungus was *Rhizopus* sp. (6.15 cfu/ml) followed by *A. terreus* (4.70 cfu/ml) > *A. niger* (3.60 cfu/ml). Similarly, at 10<sup>-4</sup> dilution level, the most abundant fungus was still *Rhizopus* sp. (4.90 cfu/ml) followed by *A. niger* (3.50 cfu/ml) (Table 4). Although the number of cfu at the highest dilution level of 10<sup>-5</sup> was comparatively lower than the lower dilution levels, the most abundant fungus at 10<sup>-5</sup> dilution level was *Rhizopus* sp. (3.65 cfu/ml) followed by *A. niger* (1.30 cfu/ml) (Table 4). However, the

intra-species difference between the number of cfu/ml of *Rhizopus* sp. at different dilution levels of 10<sup>-3</sup> and 10<sup>-4</sup> were not significant ( $P > 0.05$ ) but comparatively, the differences between the number of cfu at 10<sup>-3</sup> and 10<sup>-4</sup> dilution levels of different species: *Rhizopus* sp., *Penicillium* sp. and *Fusarium* sp. were significant ( $P < 0.05$ ) (Table 4).

#### Identification of sources and abundance of fungal isolates with entomopathogenic potential and their sources

The abundance of four fungal isolates with records of potential pathogenicity on other organisms: *Trichoderma* sp., *Penicillium* sp., *B. bassiana* and *A. niger* at different concentration levels and their sources in the University of Ibadan are presented in Tables 5 to 8. The best source for *Trichoderma* sp. as depicted by significantly higher number of cfu/ml was the PYTP site followed by the CRG (Table 5). The number of colony forming units of *Trichoderma* sp. at the different soil dilution levels varied and was highest ( $P < 0.05$ ) in the soil sample from PYTP (6.50 cfu/ml) at 10<sup>-3</sup> dilution level compared to the other



**Table 6.** Abundance of *Penicillium* sp. in soil samples from different sites at different dilution levels.

Parameter	Mean abundance/ dilution level (cfu/ml) (n=4)			LSD <sub>(0.05)</sub>
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	
PYTP	0.75±0.15	2.25±1.14	0.00±0.00	1.00
Cow stead	2.00±0.56	0.00±0.00	0.75±0.25	0.92
Piggery	2.50±1.00	1.25±0.47	0.25±0.10	1.42
Poultry	3.00±2.05	2.50±0.78	0.50±0.02	1.98
Crop garden	3.00±0.95	2.50±1.40	0.00±0.00	1.83
LSD <sub>(0.05)</sub>	1.20	0.50	0.70	

**Table 7.** Abundance of *Beauveria bassiana* in samples from different locations at different dilution levels

Parameter	Mean Abundance/ dilution level (cfu/ml) (n=4)			LSD <sub>(0.05)</sub>
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	
PYTP	2.25±0.72	2.00±0.78	1.25±0.10	1.83
Cow stead	1.00±0.10	1.25±0.95	2.25±0.95	1.00
Piggery	16.25±4.36	12.75±3.98	8.50±2.30	14.17
Poultry	6.50±2.89	7.25±2.58	6.25±2.78	6.67
Crop garden	0.00±0.00	0.25±0.10	2.25±0.96	0.83
LSD <sub>(0.05)</sub>	6.15	4.90	3.65	

**Table 8.** Abundance of *Aspergillus niger* in samples from different locations at different dilution levels.

Parameter	Mean Abundance / dilution level (cfu/ml)			LSD <sub>(0.05)</sub>
	10 <sup>-3</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	
PYTP	5.00±3.10	0.00±0.00	0.00±0.00	1.67
Cow stead	6.00±2.45	8.50±2.96	0.50±0.25	5.00
Piggery	2.50±1.20	6.00±3.95	4.50±1.60	4.33
Poultry	1.50±0.95	2.25±1.00	4.20±1.20	2.70
Crop garden	4.50±2.10	3.00±1.00	1.50±1.00	3.00
LSD <sub>(0.05)</sub>	3.60	3.50	1.30	

cfu - Colony forming Units.

sites (Table 5). Similarly, the cfu/ml of *Trichoderma* sp. was also higher at 10<sup>-4</sup> and 10<sup>-5</sup> dilution levels in the soil samples from the PYTP than the cfu on other sites. *Trichoderma* sp. was not detected in all soil samples from the piggery unit; but was detected at 10<sup>-3</sup> only in the soil sample from the Cowstead. The abundance of *Penicillium* sp. in soils from different sites at also varied significantly at the different dilution levels. *Penicillium* sp. was detected and isolated from all the soil substrates (Table 6) but mean abundance in cfu/ml was comparatively lower than the cfu of *Trichoderma* sp. from

all the sites. At 10<sup>-3</sup>, *Penicillium* sp. was most abundant in soil samples from both the Poultry unit (3.00 cfu/ml) and CRG (3.00 cfu/ml). However, the fungus was not found in the soil samples from the Cowstead at 10<sup>-4</sup> and in the soil samples from the PYTP and CRG at 10<sup>-5</sup>. *B. bassiana* was detected and isolated from all the soil samples although significantly highest in the sample from Piggery at all dilution levels: 16.25 cfu/ml at 10<sup>-3</sup>; 12.75 cfu/ml at 10<sup>-4</sup> and 8.50 cfu/ml at 10<sup>-5</sup> compared to other sites (Table 7). Interestingly, the occurrence of *A. niger* recorded in the soil samples from poultry site increased

with the dilution level from 1.50 cfu/ml at  $10^{-3}$  to 2.25 cfu/ml at  $10^{-4}$  to 4.20 cfu/ml at  $10^{-5}$ . However, the number of cfu/ml of *A. niger* (6.00) was significantly highest at  $10^{-3}$  dilution level in the sample from the Cowstead site followed by PYTP (5.00) > Crop garden (4.50) > Piggery (2.50) > Poultry (1.50).

## DISCUSSION

This study has demonstrated the possibility of obtaining local strains of entomopathogenic fungi with potential for adoption for the management of *M. vitrata* on cowpea and other insect pests of cowpea or other crops as well. Although the most abundant fungi found in this study irrespective of the sources were *Rhizopus* and *Fusarium* species, the occurrence of the other fungi with entomopathogenic or pesticidal potential especially *B. bassiana*, *Penicillium* sp. and *Trichoderma* sp. could also be readily obtained locally. This suggests that many pests especially insects could be easily managed with the well adapted local strains of entomopathogenic pathogens either singly or in an integrated pest management programme if properly harnessed (Sapna et al., 2010). Several studies had indicated and confirmed the effectiveness of entomopathogens especially *B. bassiana* and *Trichoderma* spp. as effective for control of several crop insect pests (Hajek and St. Leger, 1994; Ekesi et al., 2002; Balogun and Fagade, 2004; Enrique and Alain 2004; Fan et al., 2007; Vega et al., 2008). This study has also revealed that the PYTP, piggery and the CRG soils among others had the highest concentration of the entomopathogens – *B. bassiana*, *Penicillium* sp. and *Trichoderma* sp. This suggests that these potential entomopathogenic fungi were most active and commonly found in cropped soils rather than on the soils with decayed organic materials like the wastes from the poultry and Cowstead. The reason for the comparatively low abundance of the potentially entomopathogenic fungi on the other soil samples could be due to the lethal effects on the fungi caused probably by the heat generated in the process of decomposition of the organic wastes and formation of organic acids. It is known that most entomopathogenic fungi have a wide range of temperature tolerance (0-40° C) for reproduction and survival. However, the temperature optima for general infection and survival, mycelium growth and sporulation are usually more restricted (Lacey et al., 2001; Luangsa-ard et al., 2005).

For an entomopathogen to be considered successful as a biocontrol agent, such will require among other important traits, a predictable performance under challenging environmental conditions such as found in Nigeria (Luangsa-ard et al., 2005). The occurrence and abundance of the potentially entomopathogenic fungi detected in this study especially *B. bassiana* and

*Trichoderma* sp. as depicted by their comparatively high abundance and occurrence is known to be a major factor determining the effectiveness of entomopathogens under field conditions. It is known also that spore production characteristics of any entomopathogenic fungus are an important feature for selection as biocontrol agents against insect pests (Goettel et al., 1997). Therefore, for continuous survival of these entomopathogens in nature, there must be successful spore dissemination and this would require the production of abundant reproductive structures under advantageous environmental conditions. In this study, *Beuaveria bassiana* showed an average conidial production of  $1.65 \times 10^3$  per ml. Although the effects of growth rates on conidial production under the Nigerian climate were not part of this study, the possibility that conidial production potential may have a direct relationship to growth rates is speculated (De Cross et al., 1999). It is known also that the important factors that could significantly influence spore production especially by entomopathogens are light (Hajek and St. Leger, 1994; Butt, 2002; Sa'nchez-Murillo et al., 2004) and culture age (Edelstein et al., 2005) and these must be considered in order to optimize the conidial production. Our findings in this study also show that these entomopathogenic fungi could be cultured relatively easily in the laboratory on common solid media. These features make the fungi to be a promising candidate for incorporation into an integrated pest management programme.

## Conclusion and Recommendation

This study has shown that the available local biota could be harnessed for management of local pests. The most common entomopathogens with known potential for management of field pests of crops encountered in this study was *B. bassiana* and *Trichoderma* sp. Although *B. bassiana* was not detected on the dead larvae of *M. vitrata* in this study which may preclude any presumption about its potential for inclusion as biocontrol agent against *M. vitrata*; yet literature abound on its effectiveness against other insect pests (Gottwald, and Tedders, 1984; Feng et al., 1994; Hallsworth and Magan, 1999; Enrique and VEY, 2004; Fan et al., 2007; Tefera and Vidal, 2009; Sapna et al., 2010; Mohammadbeigi and Port, 2013) and so, its detection in the local soils is indicative of its ready availability within the local agroecosystem. Also, this study has also shown the occurrence and abundance of these fungi on actively cropped soils rather than on soils from farm yard organic materials from poultry, piggery or the cowstead. However, further work would be required to assess the effectiveness of these locally sourced potential biocontrol agents against local pests of cowpea especially *M. vitrata* under the screen house and field conditions.

## Conflict of interest

The authors did not declare any conflict of interest.

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Full Length Research Paper

## Adult emergence percentage from irradiated fruit flies, *Bactrocera zonata* and *Bactrocera cucurbitae* pupae

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Fruit flies are regarded as one of the most devastating pest of fruits and vegetables on earth planet. Generally chemical control is implemented for their control but it poses lot of eco-environmental concerns so the emphasis is now turning towards eco-friendly management practices. Bio-control is an efficient and environmentally sound approach and augmentation is primarily focus on classical biological control program. In this study, eight sub-sterilizing doses of 0, 20, 30, 40, 50, 60, 70 and 80 were tested against *Bactrocera zonata* and *Bactrocera cucurbitae* pupae. The results showed that radiation prolong the duration of pupal stage and hatching is reduced by applying radiation. This also shows that when the quantity of the radiation increases, the adult emergence decreases. This study could be very useful in exploiting the potential host for longer period of time for culturing their pupal parasitoids.

**Key words:** Sub sterilizing doses, radiation, fruit flies and emergence.

### INTRODUCTION

Fruit flies are of significant economic importance as pest in many of the important fruits. These are controlled generally by applying pesticides but these pesticides cause lot of environmental concerns and also on human health (Gill and Garg, 2014) moreover the fruit flies have attain resistance against pesticides (Van Steenwyck et al., 1975) so the focus is now diverting towards other control practices in which sterilization using radiation is an important tool. Nuclear techniques are already being practically applied convincingly in various areas of entomology (Bakri et al.,

2005). These are used against the different insect pest for suppressing the activity of insects (Faruki et al., 2005). The immature stages of the insect are most likely vulnerable to radio activity (Tilton and Brower, 1983). Additionally, radiation can be applied to semi- or completely sterilize hosts or prey for deployment in the field to increase the initial survival and build-up of natural or released biological control agents in advance of seasonal pest population build-up (Hendricks et al., 2009). By applying radiations the emergence of adults decreases (Faruki et al., 2007). It is

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also reported that emergence of black cutworm at different radiation doses applied on egg stage decreases the hatching and induced sterility in adults, more over it prolong the larval and pupal stage of the insect (Salem et al., 2014). The objective of current is to evaluate the emergence of adult fruit flies by irradiating the pupae at different doses.

## MATERIALS AND METHODS

### Adult emergence percentage of *B. zonata* and *B. cucurbitae* from the one day old pupae irradiated at different doses

The experiment was conducted at fruit flies rearing laboratory, Nuclear Institute of Agriculture (NIA), Tandojam, Pakistan. The pupae of two fruit fly species, *Bactrocera zonata* and *B. cucurbitae* were obtained from their respective colonies being maintained in NIA at temperature  $27\pm 2$  and relative humidity about 40-45% for the last several years. Then these pupae were radiated from Nuclear Institute of Medicine and Radiopathy (NIMRA) Jamshoro which is about 60 km from NIA by using gamma radiation source of Atomic Energy of Canada Limited Co-60 Ottawa, Ontario, Canada having Model GWXJ80 with dose rate of 1.20 Gy per minute. Radiations were applied at different doses ranging from 20 to 80 Gy to one day old pupae to check the emergence of fruit flies adults. The pupae irradiated at different doses and of two different species are kept in petri dishes separately then calculate the emerged adults from the pupae, meanwhile the un-emerged and half emerged pupae are also calculated. Data analyzed statistically using variance followed by DMRT test by using statistical software statistix 8.1.

## RESULTS

### Adult emergence percentage of *B. zonata* and *B. cucurbitae* from the one day old pupae irradiated at different doses

The emergence of the fruit flies, *B. zonata* and *B. cucurbitae* was affected significantly with increase in radiation doses applied (Table 1). The emergence percentage of *B. zonata* and *B. cucurbitae* was at its peak 83.25 and 87.50, respectively when no dose of radiation was applied to the pupae. Among different radiation doses, the treatment of 20 Gy resulted in highest emergence percentage, 78% of *B. zonata* and 84.50% of *B. cucurbitae*. The emergence percentage decreased gradually with the increase in radiation doses and with the subsequent doses of 30, 40, 50, 60 and 70 Gy the emergence percentage of *B. zonata* was 76.26, 72.50, 68.75, 63.75 and 60.0% while that of *B. cucurbitae* was 80.25, 76.25, 73.25, 69.0 and 62.25%, respectively. This decreasing trend in the emergence of both the fruit fly species is clearly visible in the bar series of Figures 1 and 2. Significantly the least number of adults of both species of the fruit flies (53.75 and 57, respectively) were emerged when 80 Gy radiation dose was applied to the host pupae. The present studies reflected that emergence of fruit flies is negatively correlated with the radiation doses and higher doses of

**Table 1.** Adult emergence percentage of *B. zonata* and *B. cucurbitae* from the one day old pupae irradiated at different doses.

Irradiation dose (Gy)	Emergence percentage	
	<i>B. zonata</i>	<i>B. cucurbitae</i>
0	83.25 <sup>a</sup> ± 1.93	87.50 <sup>a</sup> ± 1.71
20	78.00 <sup>b</sup> ± 0.91	84.50 <sup>b</sup> ± 0.65
30	76.26 <sup>b</sup> ± 1.38	80.25 <sup>c</sup> ± 0.65
40	72.50 <sup>c</sup> ± 0.65	76.25 <sup>d</sup> ± 1.32
50	68.75 <sup>d</sup> ± 0.63	73.25 <sup>e</sup> ± 0.85
60	63.75 <sup>e</sup> ± 0.85	69.00 <sup>f</sup> ± 0.82
70	60.00 <sup>f</sup> ± 0.91	62.25 <sup>g</sup> ± 0.85
80	53.75 <sup>g</sup> ± 1.11	57.00 <sup>h</sup> ± 1.08
LSD values	3.013	2.997

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through analysis of variance followed by DMRT (P=0.05).

irradiation applied to the pupae resulted in lower emergence percentage of the fruit fly adults. The radiation effect was comparatively higher on *B. zonata* pupae as compared to the *B. cucurbitae* as the emergence of the *B. cucurbitae* was relatively higher at all the radiation doses tested including the un-treated control (Figure 3). The results indicated a gradual decrease in the fruit fly adult emergence with the increase in the radiation doses applied.

## DISCUSSION

These studies indicated a wide range of tolerances in the usage of irradiation for rearing of the fruit fly pupal parasitoids. However, the emergence of the adult fruit flies decreased with the increasing doses of radiation. Similar results were reported by Lopez-Martinez and Hahn (2014). This may be very useful in reducing the chance of releasing the fertile fruit flies in the target areas that may have been left un-parasitized in the parasitoid rearing colony. The emergence of the adult fruit flies from the *B. cucurbitae* was comparatively higher than the *B. zonata* that may be due to the size of the pupae. As the effects of the radiation appears interrelated to the size of the pupae and the pupal size of the *B. cucurbitae* is bigger than *B. zonata* which resulted comparatively higher number of *B. cucurbitae* adult emergence at same radiation doses than *B. zonata*. Similar finding was observed by Bustos et al., (1992). Their studies provided significant support for irradiation of hosts before exposing to the parasitoids. The emergence of the parasitoids from the irradiated fruit fly pupae clearly demonstrated that the use of irradiated pupae (host) does not depict any negative effect on parasitoids. A number of studies conducted in Mexico have demonstrated a very high efficiency of the parasitoids cultured on irradiated hosts (Montoya et al., 2000).

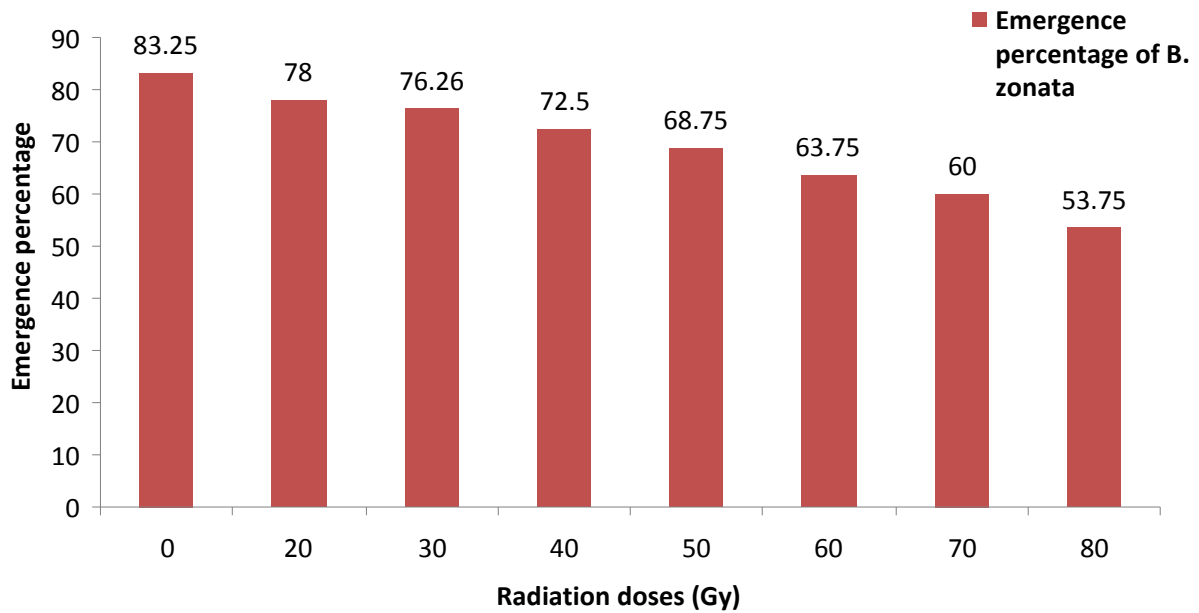


Figure 1. Emergence percentage of *B. zonata* from one day old pupae irradiated at different doses.

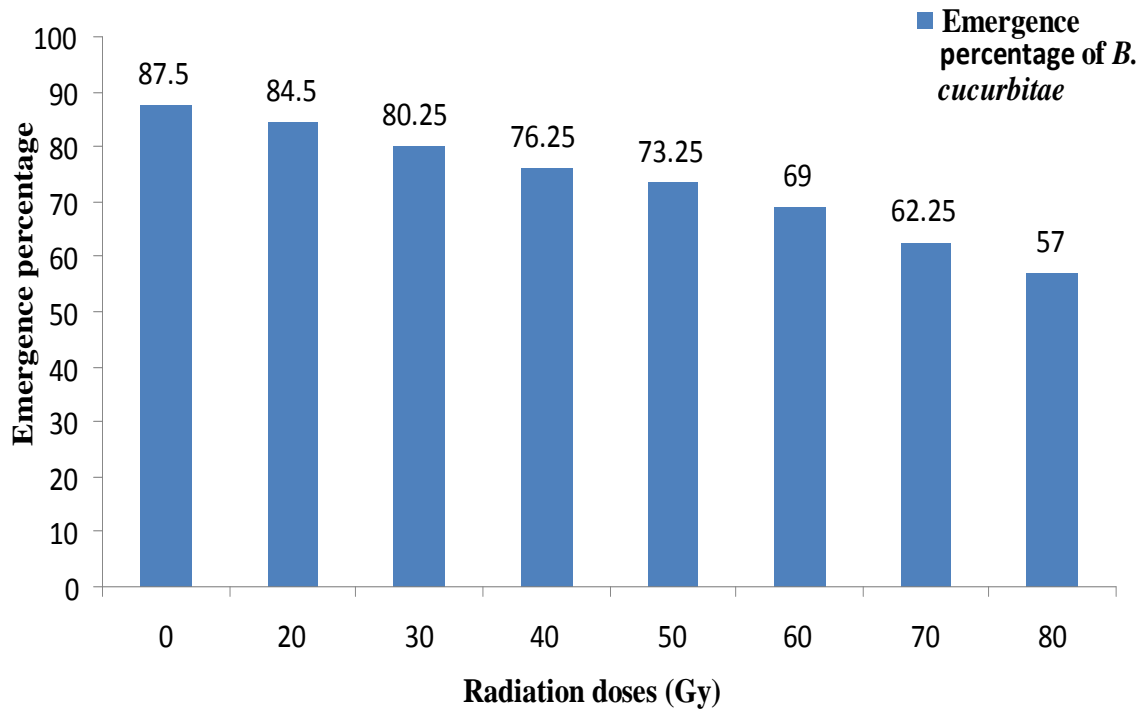


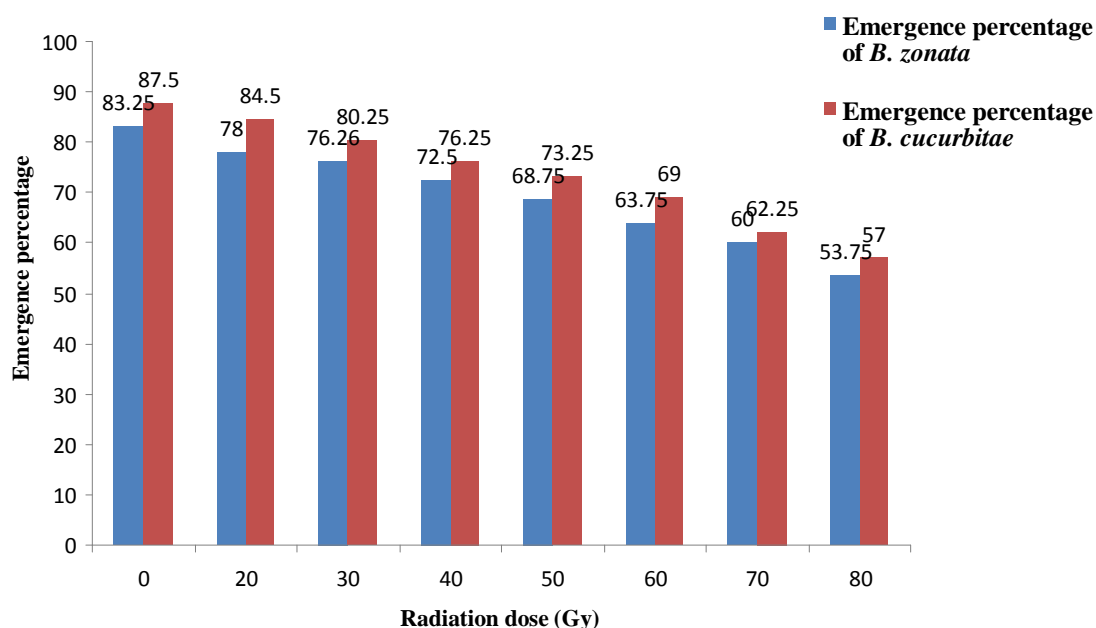
Figure 2. Emergence percentage of *B. cucurbitae* from one day old pupae irradiated at different doses.

Moreover the present studies showed higher parasitism on irradiated pupae at the dose of 40 to 50 Gy in comparison to the un-irradiated pupae.

### Conclusion

Radiations significantly decrease the emergence of the





**Figure 3.** Emergence percentage of *B. zonata* and *B. cucurbitae* from one day old pupae irradiated at different doses

host pupae of both fruit flies species and these pupae can be efficiently exploited for the rearing of bio-control agents and these pupae can be utilized for longer time period as compared with normal pupae due to slow development of the host after applying radiation.

### Conflict of interest

The authors did not declare any conflict of interest.

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