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Evaluation of Entomopathogenic Fungi against Coffee Antestia Bug, (Antestiopsis Intricate) Under Laboratory Condition at Jimma

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Abstract: *Coffee is an evergreen perennial crop grown in ecosystems, which favors insect pest to survive from year to year. Antestiopsis intricate is the number one coffee insect pest in Ethiopia and highly reduce the yield and coffee quality. The uses of bio–control agents are considered suitable alternatives to the use of chemical pesticides and the current demand for biological control products. As alternative antestia bug control two entomopathenic fungi was evaluated to know the effectiveness of these bio-control agents against this pest under laboratory condition. Greater than 65% mean mortality of antestia bugs were recorded by all tested isolates with both concentrations.*

Keywords: *Entomopathogenic fungi, Coffee and Antestiopsis intricate*

1. INTRODUCTION

Coffee is an evergreen perennial crop grown in ecosystems, which favours insect pest to survive from year to year (Wrighley, 1988). Insect pests are among the number of factors considered to limit coffee production and productivity. Among the coffee berry damaging insects, Antestia bug cause up to 9% yield loss and 48% coffee bean darkening (Million, 1988).

The uses of bio–control agents are considered suitable alternatives to the use of chemical pesticides and the current demand for biological control products has arisen in large part because of problems that has developed from the use of chemical pesticides (Dhaliwal and Koul, 2007). Of the various bio-control agents considered, the entomopathogenic fungus, *Metarhizium anisopliae* and *Beauveria bassiana* have received considerable attentions as a viable alternative to chemical pesticides.

But there is no sufficient recent information about the efficacy of entomopathogenic fungi against antestia bug under laboratory and field conditions, in Ethiopia the effectiveness of different isolation of EP fungi against antestia bug is very important as one component of integrated pest management; therefore, this experiment was initiated with the objective of evaluating the efficacy of entomopathogenic fungi against *Antesita intricata* under laboratory condition.

2. MATERIALS AND METHODS

2.1. Antestia Bug Collection and Mass Rearing

Adult coffee *Antestia intricata* was collected from Metu agricultural research sub- center. The bug collection action was done in the morning because they do not like high temperature and light from the sun and hidden in coffee canopy and difficult to collect and gain enough numbers. The collection was done by hands carefully to avoid that they may be killed. The collected bugs were put in appropriate containers (transparent bottles and perforated), and immature coffee berries were put in it in order to procure food to them.

Then after reared in Jimma agricultural research center (JARC) entomology laboratory in a glass cages (20.5x26.7x7.6 cm^3) covered with nylon mesh and providing fresh coffee twigs with large green berries for the insects at two day intervals. Fresh coffee twigs were added to cylindrical cages as feed for antestia bugs ,then the top parts of cages were covered with nylon mesh that allows air ventilation in and out easily but preventing the escape of the insect(fig below)

Fig1. *Antestia reared and spores applied at Jimma agricultural research center, entomology laboratory*

2.2. Source, Preparation of Spore Suspension and Spraying Method

Two cultured and mass reared isolates of fungal species; *M. anspolium* and *B.bassiana* used for antestia bug control in this experiment were obtained from nation Ambo plant protection research center.

The viability of spore of the fungi which collected from Ambo plant protection was checked in JARC pathology laboratory using binocular compound microscope. The concentrated fungal spore were diluted with sterile distilled water to make the spore suspension ready to be counted and 0.05% of tween 80 was added to the spore suspension to evenly distribute. The conidia suspensions and the suspension were stirred with magnetic stirrer for 4-5minute and the suspensions were filtered through sterile muslin cloth to eliminate the debris medium. The spore suspension of $1x10^7$ and $1x10^8$ conidia per ml of distilled water were prepared for each isolates by counting with haemocytometer.

The fungal isolates were sprayed by hand by using syringe and sprayed with equal quantity in each rectangular box containing these bugs needed to be sprayed and sure that the isolated fungi touch on them. The coffee berries were sprayed too.

2.3. Experimental Design and Treatments

There were seven treatments replicated three times and laid out in completely randomized design (CRD) and one control (sterile H2O) was used under the same conditions as the experimental group for comparison. Each isolates was tested at two concentrations ($1x10^7$ and $1x10^{8)}$. The treatments used were as follows;

1. PPRC-56($1x10^7$conidia/ml),
2. PPRC-56($1x10^8$conidia/ml)
3. PPRC-2($1x10^7$conidia/ml)
4. PPRC-2($1x10^8$conidia/ml)
5. MM($1x10^7$conidia/ml) and
6. MM($1x10^8$conidia/ml)
7. Control (Distilled H2O) repeated three times

2.4. Mortality of Antestia Intricata Due to Entomopathogenic Fungi

To test the efficacy of each of the fungal isolates on insects, fifth instars (nearly adults) of bugs were placed in a rectangular plastic box and sprayed with the spore solutions. To determine how many *Antestia intricata* were died without being infested by applied fungi, a control group was sprayed with only sterile water as control.

Died insect/s was counted and then transferred into a Petri dish lined with moistened filter paper and mortality due to fungi was confirmed by binocular compound microscope examination of hyphae and conidia on the surface of the dead insect (Fig. 1) in JARC pathology laboratory. The efficacy was evaluated on a daily basis for twenty days, by counting died insect which were later converted to the percent mortality. Mortality data were corrected for the corresponding control mortality by Abbott (1925) formula; CM (%) = (T%)-C (%)/100-C (%)*100

-Where, CM is corrected mortality, T is mortality in treated insects and C is mortality in untreated insects (Abbott, 1925).

2.5. Data Analysis

All data were subjected to the analysis of variance (ANOVA) with the appropriate design as per Gomez and Gomez (1984) using SAS version 9.0 computer software program (SAS, 2002). Mean separation was performed when means were significant using Least Significant Difference (LSD) at 5 % and 1% level of probability.

3. RESULTS AND DISCUSSION

Three entomopathogenic fungi isolates (from *Beavaria bassiana*, PPRC-56 and from *Metarhzium anisopleae* PPRC-2 and MM) were evaluated at different concentrations (1x107 and 1x108) rates against coffee antestia bug, which is one the major insect pest in the country. The analysis result showed that all isolates at both concentrations showed significant difference in mortality efficacy compared to check. The mortality of Antestia bug on 11^{th} day of application was not significantly different among treatments. Based on the result on 15^{th} day, PPRC-56 and PPRC-2 (both at $1x10^8$) showed greater mortality 92.59 and 85.93% respectively than others. All isolates causing more than 95% of mortality on 17^{th} days of application. (table1).

Table1. *Mean percent mortality of Antestia bug over days*

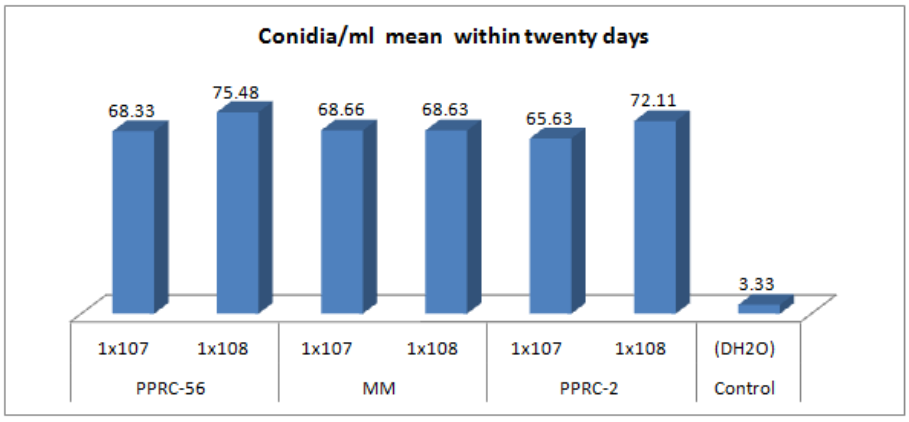
Isolates	Conidia/ml	Time after Treatment Application (Days)									
		11 day	**12 day**	**13 day**	**14 day**	**15 day**	**16 day**	**17 day**	**18 day**	**19 day**	**20 day**
PPRC-56	$1x10^{7)}$	13.3a	23.33abc	36.67bc	55.18bc	72.22bc	89.63ab	96.67a	96.30a	100a	100a
	$1x10^8$	10.0a	30.00a	46.67a	75.55a	92.59a	100a	100a	100a	100a	100a
MM	$1x10^7$	13.3a	23.33abc	36.66bc	55.18bc	75.55bc	86.30b	96.30a	100a	100a	100a
	$1x10^8$	10.0a	26.67ab	36.66bc	51.48bc	72.22bc	89.26ab	100a	100a	100a	100a
PPRC-2	$1x10^7$	10.0a	16.67c	30.00c	44.44c	68.89c	89.63ab	96.67a	100a	100a	100a
	$1x10^8$	10.0a	20.00bc	43.33ab	61.85b	85.93ab	100a	100a	100a	100a	100a
Control	(DH_2O)	0.00b	0.00(0.d	0.00d	3.33d	3.33d	3.33c	3.33b	6.67b	6.67b	6.67b
Mean		9.51	20.00	32.86	49.57	67.25	79.74	84.71	86.14	86.67	86.67
LSD(0.05)		5.4	7.64	8.54	12.42	16.30	13.04	13.62	5.71	3.82	3.82
CV (%)		32.40	21.82	14.85	14.31	13.84	9.33	5.30	3.78	2.52	2.51

Means followed by the same letters are not significantly different ($p<0.05$). DH_2O=distilled water

The analyzed results from the above table indicated applied fungal isolates were the most effective isolates for the control of coffee *Antestia intricate* under laboratory condition and produced mortality rates up to 85% after sixty day of application as compared to untreated control (distilled water sprayed) (table1).This result is correlated with the finding of Wraight and Ramos (2002), a good control means statistically significant reductions in pest numbers or damage of 75% or more, compared to an untreated control for coffee antestia bugs, *Antestiopsis lineaticolis***.** Greater than 65% mean of antestia bugs were died by all tested isolates with both concentrations under laboratory condition (graph 1).

Fig1. *Died antestia bugs due to EPF isolates*

Result from laboratory (JARC) has shown that PPRC-56 and PPRC-2 can control above 70 % mortality of antestuia bug within twenty days in higher concentration $1x10^8$ conidia per ml which means that it reduces significantly *Antestiopsis intricata* population under lab condition. Many reports are available showing that the isolates cause a high mortality on other insects (Kannan *etal*., 2008). In this experiment it has been shown that all the isolates are effective against *Antestiopsis intricata* under laboratory condition.



Graph1. *The overall mean percent mortality* ***Vs*** *the day after application*

Beavaria bassiana, PPRC-56 isolate kill antestia (75.48%) than *Metarhzium anisopleae* PPRC-2 (72.11%) *and* MM (68.63%) at $1x10^8$ concentrations. Based on this preliminary result effect of EPF isolates has the potential to kill the coffee antestia bug more effectively as compared to check (graph1) and could be used as one of integrated pest management component.

4. CONCLUSION AND RECOMMENDATIONS

EPF is an important natural biological control agent of antestia bug that significantly reduce *Antestia intricate* under lab condition and could be used as one component of integrated pest management (IPM) after evaluated at field condition. This result calls for further investigation to verify the experiment on different stages of same insect (eggs, nymph and even adults) also with these and other bio agents should be studied .Future research should be concentrated on the field evaluation and formulation of evaluated entomopathogenic fungi development for bio pesticides.

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