Efficacy of entomopathogenic fungi against cowpea weevil *Callosobruchus maculatus* (Coleoptera : Chrysomelidae)

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Introduction & Objectives

- *Callosobruchus maculatus* Fabricius 1775 (Coleoptera: Chrysomelidae) is one of the most damaging post-harvest pests of cowpea *(Vigna unguiculata* L. Walp) seeds in storage compromising their use as food and seed.
- Obsolescence of chemical control methods commonly used against this pest provides the need of sustainable alternatives in biocontrol.
- Here, we evaluate under in vitro setting, the efficacy of three entomopathogenic fungi (EPF) against *C. maculatus* using two different infection procedures (direct application through spraying and indirectly through feeding on fungal treated cowpea seeds).

Materials & Methods

Treatments

- *Beauveria bassiana* (GHA)*, Metarhizium brunneum* strains USDA 4556 and V275 (**Figure 1**)

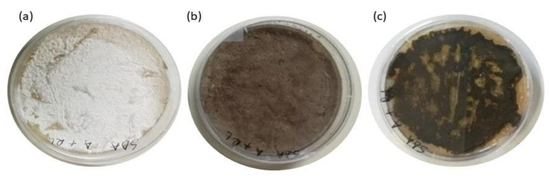


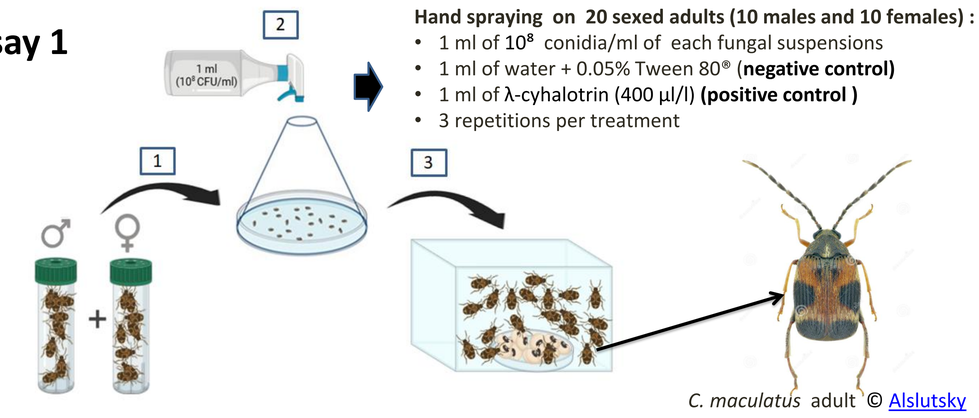
Figure 1: Sporulating EPFs strains used in bioassays against *B. rufimanus* (a) *B. bassiana* (strain GHA), (b) *M. brunneum* strain USDA 4556, and (c) strain V275 after 15 days of incubation

- Positive control : chemical insecticide "λ-cyhalotrin (400 µl/l)"
- Negative control : 0.05% Tween 80 solution

Treatments and studied parameters

- Evaluation of lethal effects (LT50 and mortality rates) on 300 sexed adults treated and 300 sexed adults exposed to treated seeds
- Evaluation of sub-lethal effects (inhibition of oviposition on seeds) on 300 sexed adults for 12 days and 300 sexed adults exposed to treated seeds on the 5th day
- Assessment of protective effect of fungal treatments applied to seeds against infestations caused to seeds by *C. maculatus* after 25 days of storage in the laboratory

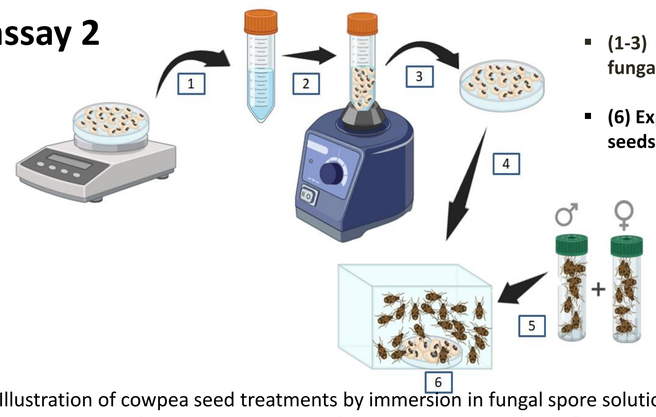
Bioassay 1



Hand spraying on 20 sexed adults (10 males and 10 females) :
- 1 ml of 10^8 conidia/ml of each fungal suspensions
- 1 ml of water + 0.05% Tween 80® **(negative control)**
- 1 ml of λ-cyhalotrin (400 µl/l) **(positive control)**
- 3 repetitions per treatment

Figure 2 : Manual spraying of CO2-anesthetized adults (1) of *Callosobruchus maculatus* using different treatments under a truncated cone (2) and placement of treated adults in boxes containing dry cowpea seeds for monitoring daily mortality and egg-laying on seeds (3). © R. Lugendo

Bioassay 2



- **(1-3) Immersion of 10 gr of seeds in 10 ml of each fungal suspensions at 10^8 conidia/ml and treatment**
- **(6) Exposure of batches of 20 sexed adults to treated seeds**

Figure 3 : Illustration of cowpea seed treatments by immersion in fungal spore solutions (1,2), vortexing the mixture (3), followed by transfer of treated seeds (4) and sexed adults of *Callosobruchus maculatus* (5) to test boxes (6) for monitoring daily mortality over 10 days. © R. Lugendo

Results

Lethal effects of three fungal strains on *Callosobruchus maculatus* adults treated and exposed to treated seeds

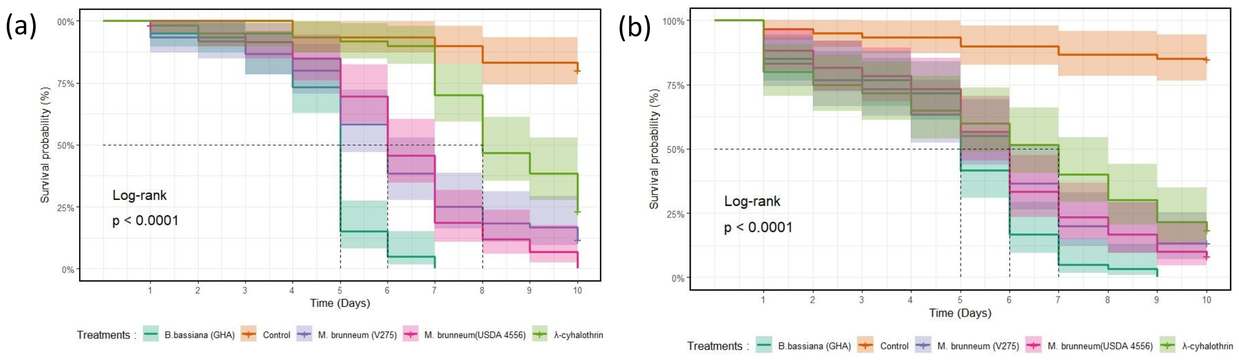


Figure 4: Survival curves of *Callosobruchus maculatus* sprayed directly with fungal suspensions (a) and exposed to treated seeds (b). The dotted lines indicate the estimated LT50 values. Each survival curve is presented with 95% confidence intervals.

Sublethal effects of three fungal strains on *Callosobruchus maculatus* adults treated and exposed to treated seeds

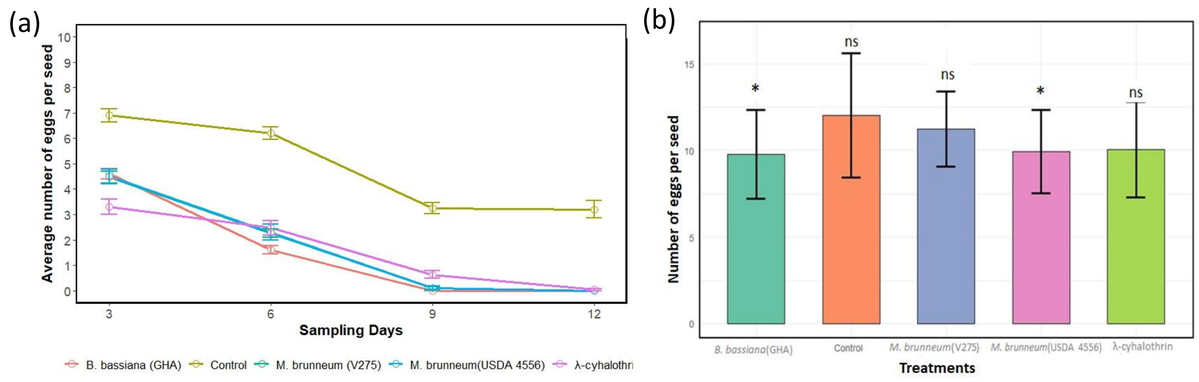


Figure 5: Evolution of *Callosobruchus maculatus* egg-laying on seeds subjected to different treatments (a) and egg-laying on treated seeds on the fifth day of exposure (b) (mean ± standard deviation of the number of eggs; *=significant; ns = not significant)

Table 1: Synthesis of EPF lethality and sublethality (egg-laying inhibition) bioassay results

				Kaplan-Meier survival estimates			Log-Rank Test Vs control			Total egg laying	Egg-laying inhibition rate
Bioassay	Observations	Spraying method	Treatments	n	Mortality observed	Median (LT_{50})	Chisq	DF	*p*		
1	LT50 +MR +EI	PD	*B. bassiana* (GHA)	60	60	5	103	1	<0,001	186 eggs	68.3 %
	LT50 +MR+ EI	PD	λ-cyhalothrin (40 mg/L)	60	46	8	36.1	1	<0,001	193 eggs	67.0%
	LT50 +MR+ EI	PD	*M. brunneum* (USDA 4556	60	59	6	92.3	1	<0,001	207 eggs	64.6%
	LT50 +MR+EI	PD	*M. brunneum* (V275)	60	53	6	65	1	<0,001	205 eggs	65.0%
	MR	PD	Tween 80 (0,05%)	60	12	>10	/	/	/	586 eggs	-
2	LT50+ MR	IS	*B. bassiana* (GHA)	60	60	5	95.4	1	<0,001	-	-
	LT50+ MR	IS	λ-cyhalothrin (40 mg/L)	60	49	7	53.7	1	<0,001	-	-
	LT50+ MR	IS	*M. brunneum* (USDA4556)	60	55	6	72.7	1	<0,001	-	-
	LT50+ MR	IS	*M. brunneum* (V275)	60	52	6	63.3	1	<0,001	-	-
	MR	IS	Tween 80 (0,05%)	60	9	>10	/	/	/	-	-

Protective effect of seed treatments against infestations of *Callosobruchus maculatus*

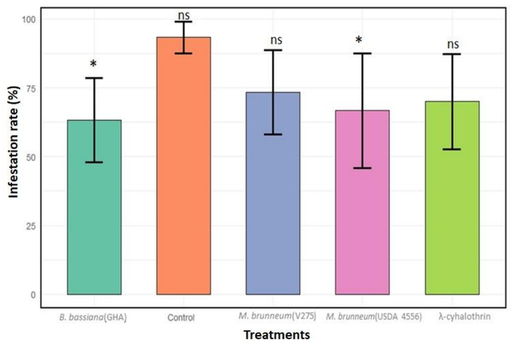


Figure 6: Seed infestation rate by *Callosobruchus maculatus* according to different treatments applied after 25 days of storage (means ± standard deviations; *= significant; ns = not significant)

Conclusions

- *Beauveria bassiana* (GHA) was proved to be more virulent, with shorter LT_{50s} of five days and the highest adult mortality rates of 100% after seven and nine days. In contrast, LT_{50} of 6 days and mortality rates of 86% to 98% were obtained after 10 days with *M. brunneum* USDA 4556 and V275 strains.
- Fungal treatments applied to cowpea seeds provided partial protection by reducing the rates of seed infestation by *C. maculatus* by 20% to 30%.
- The three fungal strains are effective entomopathogenic agents against C. maculatus and exhibit a promising alternatives to chemical pesticides commonly used in cowpea seed storage.

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