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PERFORMANCE OF *METARHIZIUM ANISOPLIAE* FORMULATIONS WITH OIL ADJUVANTS ON *TENEBRIO MOLITOR*

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SUMMARY

The aim of this experiment was to compare the effectiveness of emulsifiable oil fungal formulations to a standard water plus Tween 80 and to Peanut oil at different doses of *Metarhizium anisopliae* conidia and to observe if the emulsifiable oil content can enhance the performance of fungal conidia on *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae. Conidial suspensions were formulated in: water plus 10% Codacide[â] (rapeseed oil with emulsifiers); water plus 10% Natur'l oil[â] (rapeseed oil with emulsifiers); water plus 0.05% Tween 80 (laboratory wetting agent); peanut oil (vegetable oil). All formulations were tested at 5 different doses (0, 10^2, 10^3, 10^4, and 10^5 conidia/insect). The inoculated larvae were incubated at 25±0.5 ºC and 24 dark-hours. The average survival time for each treatment and a Log Rank statistic for significance were obtained by Kaplan-Meier Survival Analysis and the estimated LD_{50} and LD_{95} of each formulation was obtained by Probit Analysis with logit-transformed values. Emulsifiable oil fungal formulations with 10% Codacide[â] or 10% Natur'l oil[Ò] have worked as well as peanut oil. Appropriate emulsifiable oils have a great potential for mycoinsecticide formulations because they can combine the infectivity of oil based formulations with the cost effectiveness and versatility of the use of water.

INTRODUCTION

Biological control of insect-pests by fungi is potentially an important method for Integrated Pest Management. One friendly IPM tool to the environment is the biological control of insect pests using entomopathogenic fungi to avoid the chemical applications and to increase the environmental protection. For a safe and optimally targeted application with the development of the full inherent biological efficacy of the product, the active ingredient has to be formulated properly. Fungal formulation refers to the resultant composition when an entomopathogenic fungus is mixed with other ingredients. These ingredients should contribute to the viability, stability, virulence, and efficacy of the microbial control agent and the acceptance of the product by the user (1). Some inert pesticide ingredients can play an important role in improving the performance of pesticide formulations and in mediating their environmental effects. There are also some factors to be considered in the choice of fungal formulation ingredients such: cost and availability; low or no toxicity; stability, viscosity; retardation of evaporation; atomisation and suspension (2). Studies have shown that formulation in oil enhances the activity of some entomopathogenic fungi compared to conventional water-based formulations. Comparisons between the effectiveness of water-based (water + 0.01% Tween 80) and oil-based (coconut oil) formulations of *Beauveria bassiana* on adults of the cocoa weevil pest, *Pantorhytes plutus*

showed that the estimated LD_{50} of the oil formulation was 36 times lower than that for the water formulation, while the estimated value of the LD_{95} was 111 times lower (3). Studies on fungal formulations to control desert locust *Schistocerca gregaria* showed that oil-based formulations enhanced the infectivity of *Metarhizium flavoviride* at low humidities (4). These studies have been targeted against locusts, for which ultra low volume is the normal method of application. However, there is a need to adapt these findings for use with hydraulic nozzles so that entomopathogenic fungi can be used in broad acre agriculture. Fungal emulsions with the correct proportion of oil adjuvant, when sprayed with the appropriate equipment and technique, have a great potential to control insect pests in areas where the environmental conditions are favourable.

MATERIALS AND METHODS

Conidia of the *Metarhizium anisopliae* isolate code 299984 original from Trinidad were grown on SDA for 10 days at 25±0.5 ºC with 24 dark-hours. Conidia were removed using a spatula and then suspended in a total volume of 10 ml in the following formulations: water plus 10% Codacide® (oil adjuvant made by Microcide Ltd., containing 95% emulsifiable rapeseed oil), water plus 10% Natur'l oil® (oil adjuvant made by Stoller Chemicals Ltd., containing 94% pure rapeseed vegetable oil), water plus 0.05% Tween 80 (laboratory wetting agent used to facilitate suspension of hydrophobic conidia obtained from Sigma Chemicals) and Peanut oil (vegetable oil with high viscosity and very low volatility obtained from Sigma Chemicals). Conidia were mixed with adjuvants prior to the addition of water to obtain homogeneous suspensions. The stock formulation of each replicate was filtered using a sterilized muslin cloth then agitated for 3 minutes to break down conidial chains and to reduce clumping. All conidial concentrations were then calibrated in different doses (0, 10^2, 10^3, 10^4, and 10^5 conidia/insect) of *M. anisopliae* using a Neubauer's chamber. A germination test of the isolate 299984 was carried out one day before starting the bioassay. The results showed 100% of conidial viability for all samples. *Tenebrio molitor* larvae with 1.5-2.0 cm in length (60 days old) were used as model insects. Larvae were placed individually into Petri dishes of 5 cm diameter and 2 cm deep with a filter paper of 4.25 cm diameter and a small piece of fresh organic carrot. The insects were inoculated by placing 1.0 ml of suspension on the intersegmental part between the thorax and abdomen. The topical application was by a hand-operated microapplicator manufactured by Burkard Scientific. The inoculated larvae were incubated at 25±0.5 ºC and 24 dark-hours. There were a total of 20 treatments, including controls, and 4 replicates with 10 insects each. Mortality was recorded on a daily basis during the following 14 days. Cadavers were incubated at high relative humidity to confirm the infection by fungal outgrowth. The average survival time (AST) for each treatment and a Log Rank statistic for significance were calculated by Kaplan-Meier Survival Analysis using SPSS® for Windowsä (5). A Bonferroni correction was applied to the significance figures to correct for the likelihood of increased significance due to performing a number of pairwise comparisons. Each of the observed significance levels was simply multiplied by the number of pairwise comparisons before rejection or acceptance of the null hypothesis. The estimated LD_{50} and LD_{95} of each formulation were obtained by Probit Analysis. For the purpose of analysis, mortality values were transformed to logits ($L = \ln((m+0.5)/(100.5-m))$ where L is the transformation from percentage mortality (m). After that, the relative median potency of each formulation was calculated dividing the higher original LD_{50} or LD_{95} value of each formulation by the lower original LD_{50} or LD_{95} value of each other formulation.

RESULTS AND DISCUSSION

Average survival time of *T. molitor* larvae inoculated with different doses of four *M. anisopliae* formulations are shown in Table 1 and Figure 1. There were significant differences ($p<0.05$) between formulations and between doses in the same formulations. These significant differences between formulations were more pronounced in high doses. Under the doses 10^5, 10^4 and 10^3 conidia/insect formulated in water + 10% Codacide® killed more quickly than water + 0.05% Tween 80. Under the doses 10^4 and 10^3 conidia/insect formulated in water + 10% Codacide® killed more quickly than Peanut oil formulation. AST of *T. molitor* larvae inoculated with *M. anisopliae* formulated in Peanut oil was 4.25 days and it was not significantly different ($p<0.05$) from the treatment water plus 10% Codacide® (4.83 days) at the dose of 10^5 conidia/insect, but there was between Peanut oil and water plus 10% Natur'l oil® (5.95 days) at the same dose.

Table 1. Average survival time of *Tenebrio molitor* larvae inoculated with different doses of four *Metarhizium anisopliae* formulations at 25±0.5 ºC and 24 dark-hours.

Doses	Average survival time for each formulation with standard error (days)			
(conidia/ insect	Peanut oil	water + 10% Codacide	water + 10% Natur'l oil	water + 0.05% Tween 80
10^5	4.25±0.22 a A	4.83±0.17 a A	5.95±0.20 b A	6.80±0.27b A
10^4	6.82±0.40 b B	5.20±0.14 a A	6.45±0.21 b A	8.67±0.41c B
10^3	8.90±0.77 b C	7.55±0.31 a B	8.28±0.34 ab B	9.53±0.40b B
10^2	9.43±0.71 a C	12.08±0.41 b C	10.48±0.39 ab C	12.32±0.40b C
0 (control)	12.88±0.54 a D	14.00±0.00 b D	14.00±0.00 b D	14.00±0.00bD

Means followed by the same small letter within the same row, and means followed by the same capital letter within the same column are not significantly different ($p<0.05$).

There were significant differences ($p<0.05$) between different doses in all formulations. When the dose increases the AST of the insect decrease. AST of *T. molitor* larvae in the control of each formulation was significantly different ($p<0.05$) from all other doses in the same formulation.

The computed maximum likelihood regression line for each formulation and results of the estimated LD_{50} and LD_{95} 7 days after application are shown in Table 2. The regression lines of each formulation are shown in Figure 2. The relative median potency for LD_{50} and LD_{95} of each formulation are shown in Table 3. Water plus 10% Codacide® and Peanut oil were the most effective fungal formulations followed by water plus 10% Natur'l oil® formulation for LD_{50}. Water plus 10 % Codacide® was 1.3 times more effective than Peanut oil formulation, 2.9 times more effective than water plus 10% Natur'l oil® formulation, and 17.1 times more effective than water + 0.05% Tween 80. Peanut oil formulation was 2.3 times more effective than water plus 10% Natur'l oil® for LD_{50}, but it was 1.9 times less effective than that same formulation for LD_{95}. Peanut oil is working better in low doses than in high doses when compared to water plus 10% Natur'l oil®. Water + 0.05% Tween 80 was the least effective formulation (Table 3).

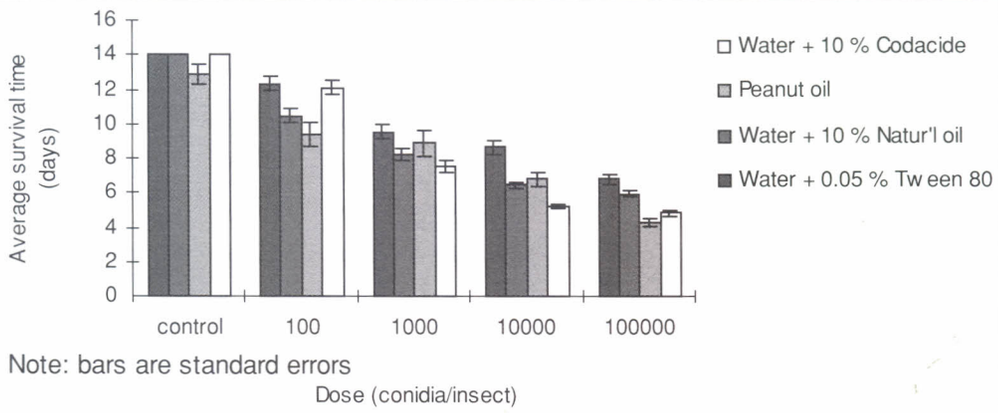

Figure 1. Average survival time of Tenebrio molitor larvae inoculated with different doses of four Metarhizium anisopliae formulations at 25 ± 0.5 º C and 24 dark-hours.

Table 2. Lethal dose estimations and original values of the estimated LD_{50} and LD_{95} of four *Metarhizium anisopliae* formulations applied on *Tenebrio molitor* larvae, after 7 days incubated at 25±0.5 ºC and 24 dark hours.

Formulation	Lethal dose estimations (in conidia/insect) Computed maximum likelihood regression line: X = log dose Y = logit mortality with R^2	Estimated LD_{50} (dose of conidia/insect)	Estimated LD_{95} (dose of conidia/insect)
water + 10% Codacide®	Y = 2.6248 X - 7.4763 R^2 = 0.99	706.64	8649.01
Peanut oil	Y = 1.0678 X - 3.1503 R^2 = 0.79	891.81	420085.51
water + 10% Natur'l oil®	Y = 1.3925 X - 4.6020 R^2 = 0.93	2017.65	226263.24
water + 0.05% Tween 80	Y = 1.3113 X - 5.3523 R^2 = 0.98	12069.09	1812882.08

The formulation clearly has an important role to play in the optimization of activity for a mycoinsecticide. The estimate of relative median potency of water + 10% Codacide® for LD_{50} was higher than all other formulations. The addition of the oil adjuvants Codacide® and Natur'l oil® in the concentration of 10% enhanced infectivity of *M. anisopliae* in water-based formulations on *T. molitor* larvae compared to water + 0.05% Tween 80. Those formulations with oil adjuvants presented estimates of relative median potency for LD_{95} higher than Peanut oil.

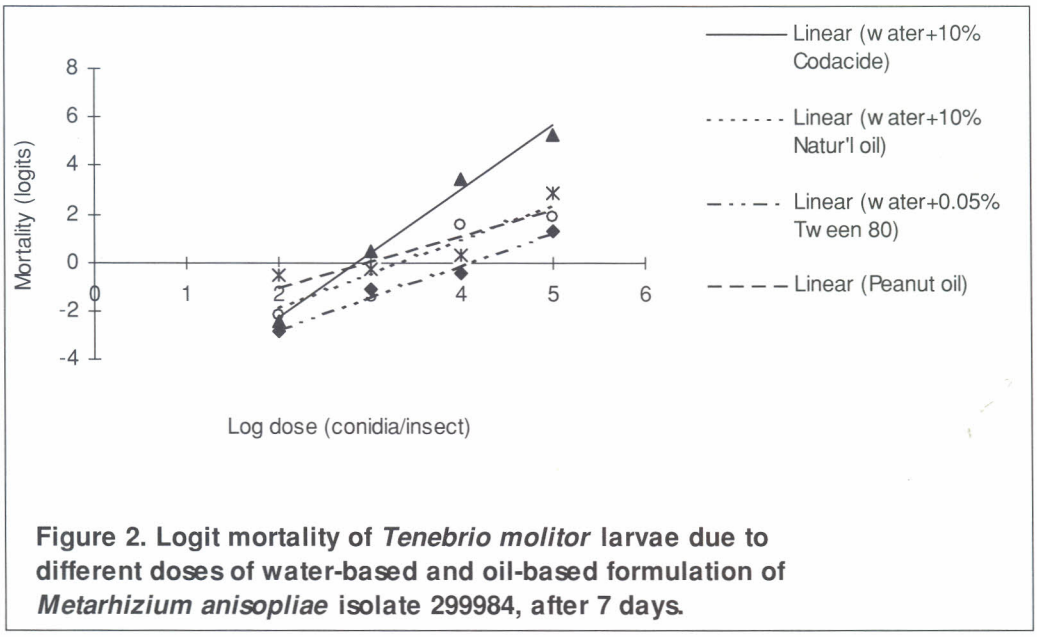

Figure 2. Logit mortality of *Tenebrio molitor* larvae due to different doses of water-based and oil-based formulation of *Metarhizium anisopliae* isolate 299984, after 7 days.

Table 3. Comparison between the estimate of relative median potency of different *Metarhizium anisopliae* formulations on *Tenebrio molitor* larvae.

Formulation A	Estimate of relative median potency of formulation A versus formulation B		Formulation B
	LD_{50}	LD_{95}	
water plus 10% Codacide	1.3	48.6	Peanut oil
water plus 10% Codacide	2.9	26.2	water + 10% Natur'l oilâ
water plus 10% Codacide	17.1	209.6	water + 0.05% Tween 80
Peanut oil	2.3	-	water + 10% Natur'l oilâ
Peanut oil	13.5	4.3	water + 0.05% Tween 80
water + 10% Natur'l oilâ	-	1.9	Peanut oil
water + 10% Natur'l oilâ	6.0	8.0	water + 0.05% Tween 80

Emulsifiable oil fungal formulations with 10 % Codacideâ or 10 % Natur'l oilÒ have worked as well as peanut oil for some reasons. Insect cuticles and fungal conidia have water repellent properties, but the oil adjuvants made of rapeseed oil are rich in triglycerides similar to the insect cuticle. When the conidia are mixed with those oil adjuvants there will be more affinity for the conidia stick to the insect cuticle. Conidia mixed with an emulsifiable oil spread on the insect cuticle further than an equivalent volume of pure water or water plus Tween 80 droplet. Therefore, the proportion of conidia in contact with the insect cuticle is higher than without oil adjuvants. Appropriate emulsifiable oils have a great potential for mycoinsecticide formulations because they can combine the infectivity of oil based formulations with the cost effectiveness and versatility of the use of water.

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