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Effects of formulation and volume application rate on the secondary pick-up of *Metarhizium anisopliae* conidia pathogenic to the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae)

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INTRODUCTION

The control of insect pests using entomopathogenic fungi to avoid chemical applications and to increase environmental protection is a desirable option (Ahmed and Leather, 1994). For a safe and optimally targeted application with the development of the full inherent biological efficacy of the product, the fungal conidia have to be formulated properly.

The addition of 10% of an emulsifiable oil enhanced infectivity of *Metarhizium anisopliae* in water-based formulation on yellow meal worm, *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Alves et al., 1998).

Infections can arise from direct impaction of spray droplets, secondary pick-up of conidia from the spray residue and via horizontal transmission of the pathogen from infected individuals (Bateman et al., 1998).

The main purpose of this investigation was to evaluate the effects of the spray residue of *M. anisopliae* var. *acridum* formulations sprayed at different volume application rates and doses by an experimental track sprayer, on the mortality of desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). Effects of the volume application rate and presence of *M. anisopliae* conidia, sprayed by a spinning disc atomiser, on the spray characteristics of an emulsifiable oil-based formulation and an oil-based formulation were also evaluated.

MATERIAL AND METHODS

EFFECTS OF FORMULATION AND VOLUME APPLICATION RATE ON THE SECONDARY PICK-UP OF *M. anisopliae* CONIDIA PATHOGENIC TO THE DESERT LOCUST *S. gregaria*.

The desert locust, *S. gregaria*, was reared in cages inside a controlled environment (CE) room (50 ± 5% R.H. and 27 ± 1 °C).

Conidia of *M. anisopliae* var. *acridum* isolate IMI 330189 were formulated in water plus 10% of an emulsifiable oil and in a mixture of two paraffinic oils (Table 1).

Table 1. Trade names, composition, and suppliers of the emulsifiable oil and mineral oils.

Trade name	Composition	Company
Codacide®	Emulsifiable oil containing 95% rapeseed oil and 5% of emulsifiable	Microcide Ltd.
Ondina EL	Refined paraffinic oil	Shell Oil Co.
Shellsol T	Refined paraffinic oil	Alcohols Ltd.

Conidial concentrations of both formulations were then calibrated using a Neubauer's chamber, to be applied at the equivalent doses and proportional volumes per hectare (Table 2).

Both control formulations with no conidia were also sprayed at 10 l/ha (blank formulations). The fungal formulations were applied on seedlings of wheat by a track sprayer designed to simulate field spraying in laboratory conditions (Bateman, 1994).

Table 2. Conidial number of *M. anisopliae* var. acridum, per millilitre applied to obtain the equivalent dose at different volume application rates.

Equivalent doses	Equivalent volume application rates (l/ha)			
(conidia/ha)	0.3	1.0	3.2	10.0
10^{10}	3.13×10^7	1×10^7	3.13×10^6	1×10^6
10^{11}	3.13×10^8	1×10^8	3.13×10^7	1×10^7
10^{12}	3.13×10^9	1×10^9	3.13×10^8	1×10^8

After each application, 20 adult locusts were placed into a sprayed cage with wheat and held for a period of 100 minutes in a CE room at 30 ± 0.5 °C, 30 ± 5% RH with a day:night regime of 14:10 h, to have contact with the spray residue of the *M. anisopliae* formulations. The insects were then housed in plastic boxes and incubated in the same CE room. Mortality was recorded daily during 14 days.

The experiment was a factorial design with two formulations, four volume application rates and three doses of conidia. There were controls for each formulation and three replicates with 20 insects each carried out on three different occasions. Factorial analyses of variance (ANOVA) of the corrected insect survival data was performed.

EFFECTS OF THE VOLUME APPLICATION RATE AND PRESENCE OF *M. anisopliae* CONIDIA, SPRAYED BY A SPINNING DISC ATOMISER, ON THE SPRAY CHARACTERISTICS OF AN EMULSIFIABLE OIL-BASED FORMULATION AND AN OIL-BASED FORMULATION.

Spraying samples of each treatment were collected to evaluate the number of droplets per cm^2 by water and oil-sensitive paper.

The droplet size spectra were assessed by spraying the formulations with a spinning disc atomiser fitted in the cabinet of a Malvern® Particle Size Analyzer to analyse the influence of both formulations without and with conidia (10^{12} conidia/ha) on droplet size, droplet distribution and droplet volume.

Volume median diameter (VMD) and relative Span are two useful statistics for describing droplet size spectra.

The droplet volume, expressed in picolitres (pl) was calculated and based on the volume represented by the VMD (Bateman et al., 1998). After that, the number of conidia per droplet was calculated and based on the droplet volume, equivalent VAR and on the dose applied (10^{12} conidia/ha). Finally, the number of conidia per cm^2 was calculated timing the number of conidia per droplet by the number of droplets per cm^2.

Data factorial analyses of variances (ANOVA) of VMD, Span, droplet volume, number of conidia per droplet, number of droplets per cm^2 and number of conidia per cm^2 were performed using the statistical package SPSS® for Windows™.

RESULTS

EFFECTS OF FORMULATION AND VOLUME APPLICATION RATE ON THE SECONDARY PICK-UP OF *M. anisopliae* CONIDIA PATHOGENIC TO THE DESERT LOCUST *S. gregaria*

Comparisons of mean proportional survival of *S. gregaria* infected by the spray residue of the two conidial formulations at different doses are shown in Figure 1.

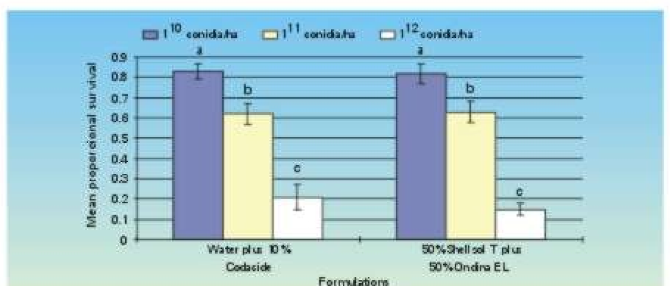

Figure 1. Mean proporcional survival (± s.e) of *S. gregaria* infected by the spray residue of *M. anisopliae* var. *acridum* conidial formulations applied on wheat seedlings under different doses, after 14 days.

Note: Means followed by the same letter are not significantly different ($p < 0.05$).

The results of the comparison between the emulsifiable oil conidial formulation (10^{12} conidia/ha) at different VARs are shown in Figure 2 and for the oil-based formulation in Figure 3.

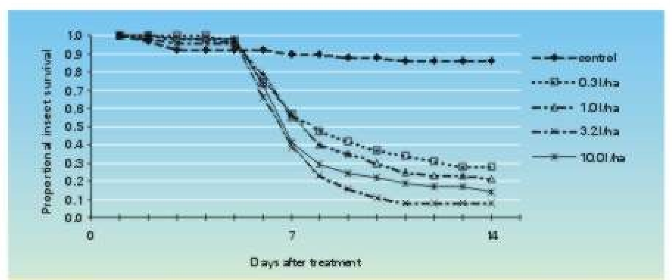

Figure 2. Proportional survival of the desert locust, *S. gregaria*, infected by the spray residue of *M. anisopliae* var. *acridum* formulated in water plus 10% Codacide at the dose of 10^{12} conidia/ha and sprayed at different volume application rates.

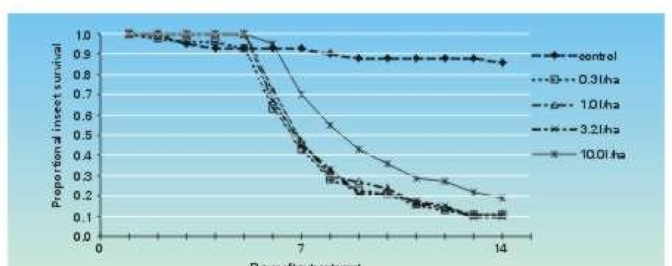

Figure 3. Proportional survival of the desert locust, *S. gregaria*, infected by the spray residue of *M. anisopliae* var. *acridum* formulated in 50% Shellsol plus 50% Ondina at the dose of 10^{12} conidia/ha and sprayed at different volume application rates.

EFFECTS OF THE VOLUME APPLICATION RATE AND PRESENCE OF *M. anisopliae* CONIDIA, SPRAYED BY A SPINNING DISC ATOMISER, ON THE SPRAY CHARACTERISTICS OF AN EMULSIFIABLE OIL-BASED FORMULATION AND AN OIL-BASED FORMULATION

ANOVA on VMD revealed significant differences between formulations and between VARs formulations, but there were no significant differences between droplet sizes with and without conidia.

There were significant differences between VARs for the relative Span (Table 3). Droplet volume was significantly affected by formulations and VARs (Table 3).

The number of conidia per droplet was significantly affected by VARs. It depends on the droplet volume and the dose applied (Table 3).

The number of droplets/cm^2 was significantly affected by formulations and VARs. When the VAR increased, the number of droplets/cm^2 also increased (Figure 4).

The locusts were infected by an estimated number of conidia per cm^2 (Figure 5) on the wheat seedlings and it was significantly affected by formulations.

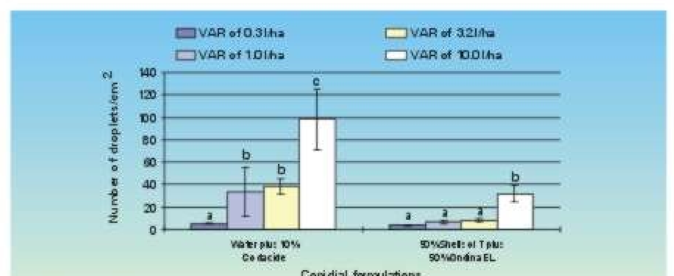

Figure 4. Number of droplets per cm^2 (± s.e) of two conidial formulations sprayed at different VARs with a spinning disc atomiser working at 6030 rpm and counted on water and oil-sesitive papers.

Note: Means followed by the same letter within the same formulation are not significantly different ($p < 0.05$).

Table 3. Effects of the equivalent volume application rate on the spray characteristics of an emulsifiable oil-based formulation and an oil-based formulation.

Formulation	Flow rate (ml/min)	Peristaltic pump speed setting	Equivalent VAR (l/ha)	VMD with standard error (µm)	Relative Span with standard error	Estimated droplet volume with standard error (pl)	Estimated number of conidia per droplet with standard error
Water plus 10% Codacide	0.5	9	0.3	87.69 ± 20.18 bc	2.38 ± 1.24 a	471.44 ± 299.59 ab	1473.28 ± 936.23 a
	1.5	30	1.0	77.35 ± 0.40 c	0.40 ± 0.04 c	242.35 ± 3.77 b	242.35 ± 3.77 bc
	4.8	94	3.2	107.63 ± 2.62 ab	0.63 ± 0.11 c	655.13 ± 46.63 a	204.73 ± 14.57 bc
	15.1	99	10.0	110.49 ± 3.48 a	0.70 ± 0.10 c	710.44 ± 65.38 a	71.04 ± 6.54 c
50% Shellsol T plus 50% Ondina EL	0.5	14	0.3	67.91 ± 4.22 c	4.43 ± 0.66 b	167.87 ± 32.57 b	524.58 ± 101.77 ab
	1.5	45	1.0	73.48± 0.75 c	0.33 ± 0.10 c	207.83 ± 6.32 b	207.83 ± 6.32 bc
	4.8	71	3.2	75.19± 0.70 c	0.49 ± 0.13 c	222.66 ± 6.22 b	69.58 ± 1.94 c
	15.1	90	10.0	80.81± 1.52 c	0.75 ± 0.02 c	279.82 ± 15.42 b	27.98 ± 1.54 c

Means followed by the same letter within a same column are not significantly different ($p < 0.05$).

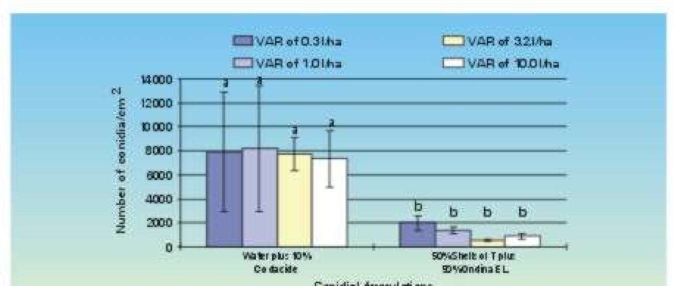

Figure 5. Estimated mean number of conidia per cm^2 (± s.e) of two conidial formulations sprayed at different VARs with a spinning disc atomiser working at 6030 rpm.

Note: Means followed by the same letter are not significantly different ($p < 0.05$).

DISCUSSION

The insects were not hit directly, but they became infected by the spray residue of the conidial formulation which had impacted on the foliage.

The most effective dose was 10^{12} conidia/ha for both formulations and this dose is very similar to the dose applied in the field by different authors (Bateman et al., 1994; Lomer et al., 1993). Therefore, the spray residue on foliage had an amount of conidia sufficient to infect and to reduce the desert locust population in the experimental conditions by secondary pick-up.

The emulsifiable oil conidial formulation presented the VMD for all equivalent VARs in the right size-band 75-150 µm suitable for water-based controlled droplet application (CDA). The oil-based formulation also had all droplet sizes in the appropriate size-band 50-100 µm (Bateman, 1993) for all equivalent VARs. These results can help to explain why there were not significant differences between the proportional survival of *S. gregaria*, when the formulations were applied at different VARs.

Both conidial formulations presented uniform droplet distributions with characteristics of CDA sprays when applied at 1, 3.2 and 10 l/ha. A good droplet coverage on foliage is very important to control insect pests in the field. Locusts are very mobile insects and maybe this was another reason why all VARs with different Spans have worked equally at the same doses.

About the droplet density, the peristaltic pump was sending more liquid for high VARs than for low VARs, but the spinning disc atomiser was working at the same rotation per minute (6030) all the time. Then, the liquid from an equivalent VAR, 10 l/ha, was broken into a number of droplets higher than for other, smaller VARs for both formulations. The difference between formulations could be caused by the difference of quality between the water-sensitive paper and oil-sensitive paper. The water-sensitive paper is more easily sensitised by water-based formulations than the oil-sensitive paper by oil-based formulations, but comparisons between the number of droplets/cm^2 within the same formulations could be done without problems.

The number of conidia per cm^2, for a given dose, is dependent on the number of droplets per cm^2 and on the number of conidia per droplet. When the VAR increased, the number of droplets per cm^2 increased and the number of conidia per droplet decreased. At the end, the number of conidia per cm^2 was not significantly different within the same formulation when applied at different VARs.

The emulsifiable oil conidial formulation worked as well as the oil-based formulation in these experimental conditions, and it has a great potential to control insect pests, when sprayed with the appropriate equipment and technique.

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