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Full Length Research Paper

Pathogenicity of local *Metarhizium anisopliae* var. *acridum* strains on *Locusta migratoria migratorioides* Reiche and Farmaire and *Zonocerus variegatus* Linnaeus in Senegal

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Locusts and grasshoppers are the most important economical threat in the sahelian agricultural system. Principal control strategies of these pests are synthetic chemicals which are, however, harmful to the environment and human health. *Metarhizium anisopliae* based biopesticide Green Muscle IMI330189 has been recently developed for the control of locusts and grasshoppers. In this paper, we assessed the pathogenicity of three local strains of *M. anisopliae* var. *acridum* on *Locusta migratoria migratorioides* and *Zonocerus variegatus*, in comparison to the commercial product, IMI330189. There was various level of pathogenicity within the strains on the two pests. On *L. migratoria*, DPV5 caused the highest mortality after three weeks (91.2%). There was a significant difference between DPV5 and IMI330189. However, there were no significant differences between IMI330189 and the other strains. On *Z. variegatus* there were no significant differences between IMI330189 DPV5 and DPV10. DPV15 had the lowest pathogenic activity. Moreover, the comparison of susceptibility of both insect pests to *Metarhizium* strains showed that *L. migratoria* is more susceptible than *Z. variegatus*. DPV5 had the shortest LT_{50} on *L. migratoria* 7.1 days whereas on *Z. variegatus*, the LT_{50} value of DPV5 was comparable to IMI330189. Our study suggests that, DPV5 which was isolated from *Kraussaria angulifera* in Senegal is a promising candidate for future development for locusts and grasshoppers control in the country.

Key words: Green Muscle, African migratory locust, grasshoppers, local strains, biopesticides.

INTRODUCTION

The African migratory locust *Locusta migratoria migratorioides* and the variegated grasshopper *Zonocerus variegatus* (Orthoptera: *Acrididae*) are among the most damaging pest in the Sahel (Lomer et al., 2001). Late instars and adults cause economic damage directly to pastures and crops such as millet, maize, beans, sweet potato, cassava and vegetable (Amatobi et al., 1986). In 2007, the total cost of the campaign was estimated at 400 million US$, of which 280 million were used for control operations and 90 million for food assistance and rehabilitation of communities affected by the upsurge (FAO, 2007). Control strategies of these pests are classically based on the use of chemical pesticides. In

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2004, over 300,000 ha were treated with synthetic chemical costing around $1,550,000 and 4,301,000 for humanitarian and disaster assistance (USAID, 2004). Farmers mostly rely on chemical insecticides such as fenitrothion and malathion for emergency situation. Although effective in reducing the pest population and their incidences, pesticides affect the potential natural enemies. Pesticides are also involved in environmental and health issues (risks of intoxication to farmers and consumers) (Peveling, 2001). The concern about environmental and toxicological issues of chemical control of locusts and grasshoppers has stimulated interest in the development of biological control based on entomopathogens (Chandler et al., 2008; Kooyman, 2003).

The *Metarhizium anisopliae* var. *acridum* (Driver et al., 2000) strain IMI 330189 previously isolated from *Ornithachris cavroisi* in Niger in 1993, has been developed as Green Muscle for the control of locust (Greathead et al., 1994; Lomer et al., 2001). Accordingly, *Metarhizium* has been recommended by the Food and Agriculture Organisation (FAO), Pesticide Referee Group for locust control in environmentally sensitive areas. Most significantly, Green Muscle™ has recently undergone successful registration for locust control in South Africa (Thomas, 2000) and in Senegal. The advantage of this biopesticide in locust control is its safety regarding to natural enemies and human health (Douthwaite et al., 2001). Actually, Green Muscle is widely applied in Africa mainly for the control of the desert locust *Shistocerca gregaria* (Van der Valk, 2007) and the Senegalese grasshopper (Douro-Kpindou et al., 2008; Lomer et al., 1999). However, the pathogenicity of this biopesticide on other species of locusts and grasshoppers has been poorly studied. Besides, during the LUBILOSA (Lutte Biologique contre les Locustes et les Sauteriaux), surveys were conducted, in order to contribute in finding potential local strains for locust and grasshoppers control. Over 30 *M. anisopliae* strains were collected from locust and grasshoppers, in different locality of Senegal. Purification and genetic identification as *M. anisopliae* var. *acridum* was done by USDA-ARS (Bon et al., 2003). Very few attempts on the entomopathogenic potentiality have been investigated or published so far. Therefore, the aim of this work was, (1) to assess the pathogenicity of three local strains DPV5, DPV10 and DPV15 on *L. migratoria migratorioides* Reiche and Fairmaire and *Z. variegatus* Linné; (2) to compare their performance with the commercial strain IMI330189.

MATERIALS AND METHODS

Locusts and grasshoppers colony

A colony of *L. migratoria migratorioides* and *Z. variegatus* was maintained at the laboratory of Agricultural Entomology DPV, Senegal. Insect were reared in cages containing sterile soil for ovoposition. Wheat leaves and bran were provided as food source. Eggs were collected from the sterile soil and placed in an incubator to allow eggs to hatch at 25 ± 1°C. The L_3-L_4 larval instars were used for bioassay.

Bioassay

Local strains of *M. anisopliae* var. *acridum*

Three local strains of *Metarhizium* DPV5, DPV10 and DPV15 previously isolated from Senegal (Table 1), were selected based on previous pathogenicity studies and no bioassay has been attempted on *Z. variegatus* and *L. migratoria*. The Green Muscle strain IMI330189 was purchased from CABI.

Preparation of inoculum

A 10 ml suspension of 10^6 conidia/ml of each strain was spread plated on Sabouraud Dextrose agar plates and placed in the incubator at 25 ± 1°C for 10 days after which the conidia were harvested by scrapping using a sterile spatula. Inoculative suspensions were prepared by mixing conidia with distilled water and Tween 80. Appropriate concentrations were estimated using a Neubauer hemacytometer and serial dilution (Goettel and Inglis, 1997). A standard concentration of 1.2 10^7 conidia ml^{-1} was used for all the treatments. A control treatment of distilled water and Tween without fungus was included.

Germination assessment

A malt-agar media was prepared to assess the percentage of germination of the conidia of each strain including the Green Muscle, to assess the viability before the bioassay. A suspension of 10^6 conidia /ml was prepared and 0.1 ml was spread plated on malt-agar and placed in the incubator for 16 h. 4 cover slips on each plate and the germination percentages were estimated according to (Lubilosa, 1999).

Inoculation and bioassay

A topical inoculation was applied on each insect. A quantity of 0.2 ml was applied on the insect pronotum using a micropipette. Each treatment was replicated 3 times and each replicate having 20 insects. However, in each replicate the insects were further grouped and observed in batches to avoid cannibalism and escapes. Wheat leaves and bran were provided as food source, insects were kept in a room in 12:12 D: L conditions at 25 ± 1°C. Bioassay containers were cleaned daily and mortality was monitored for three weeks. Dead insects were removed and observed under microscope for mycosis.

Data analysis

Mortality data were pooled altogether and subjected to SAS (SAS, 2003). A probit analysis was used to determine the LT_{50} and fiducial limits. A general linear model procedure (GLM) was used and means were separated using a Student and Newman Keuls test (SNK). As for the comparison between insect susceptibility a t-test was performed. The level of significance was kept at 5% for all data analysis.

Table 1. Origins and germination percentage of the selected *M. anisopliae* strains.

Strain	Origin	Isolation	Germination (%)
DPV5	MBegué (Kaffrine)	*K. angulifera*	93
DPV10	Nioro (Kaolack)	*O. cavroisi*	95
DPV15	Nioro (Kaolack)	*O. cavroisi*	96
IMI330189	Niamey	*O. cavroisi*	96

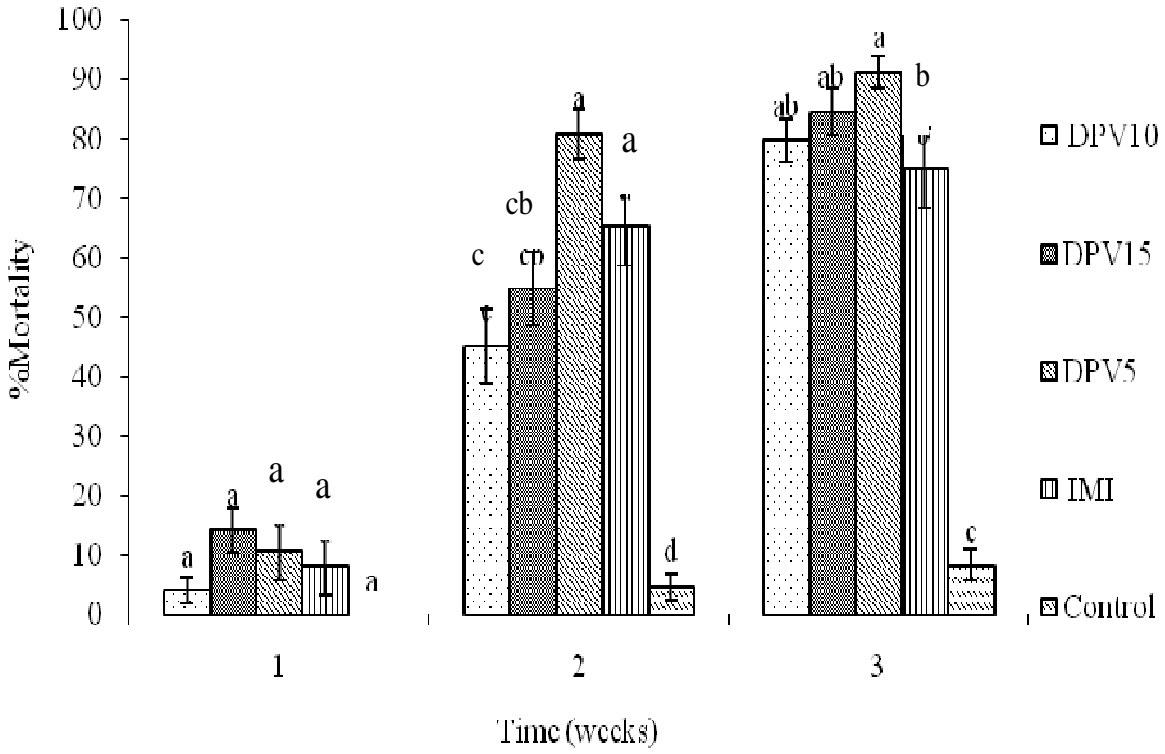

Figure 1. Cumulative mean mortality per week of *L. migratoria* infected with 3 local strains of *M. anisopliae* DPV10, DPV15, DPV5 and IMI330189. Within a week means with same letters are not significantly different by Student Newman Keuls test.

RESULTS

Effect of the *M. anisopliae* strains on *L. migratoria*

At the standard concentration, the conidia germination percentage was over 90% (Table 1). All the *Metarhizium* treatments were pathogenic to *L. migratoria*. There was no difference in terms of mortality among the treatments and control in the first week after treatment. DPV15 caused the highest mortality, while DPV10 had the lowest mortality during that same week. In the second week of observation, there was a significant difference in mortality between locusts treated with DPV5 and the rest of the treatments ($F = 28.06$; $P < 0.0001$). In the third week, there was a significant difference in the mortality of the locusts treated with DPV5 when compared with IMI330189 ($F = 63.47$; $P < 0.0001$). However, no significant difference in mortality between IMI330189 and the rest of the treatment was observed (Figure 1). Analysis of the LT_{50} values showed that, DPV5 had the shortest median lethal time 7.8 days. There is no overlapping in the fiducial limits 95% between DPV5 and the rest of the strains (Table 2). There was no significant difference of LT_{50} between IMI330189 and DPV15. DPV10 had the longest LT_{50} value when compared with other strains.

Effects of the *M. anisopliae* strains on *Z. variegatus*

Mortality recorded during the first week showed significant difference between treatments and the control except with DPV15 where mortality did not occur. There were no

Table 2. Median lethal time LT_{50} of 3 local strains of *M. anisopliae,* DPV10, DPV15, DPV5 and IMI330189 applied *L. migratoria* and *Z. variegatus*.

Strain	*L. migratoria*			*Z. variegatus*		
	LT_{50}	Fiducial limits	P	LT_{50}	Fiducial limits	P
DPV10	11.6	11.4 - 11.9	0.0001	44.2	40.3 - 49.0	0.0001
DPV15	9.5	9.2 - 9.7	0.0001	29.8	28.0 - 32.2	0.0001
DPV5	7.8	7.6 - 8.0	0.0001	27.1	25.9 - 28.4	0.0001
IMI	9.9	9.6 - 10.1	0.0001	27.0	25.8 - 28.4	0.0001
Control	49.0	41.2 - 62.6	0.0001	112.5	83.0 - 172.0	0.0001

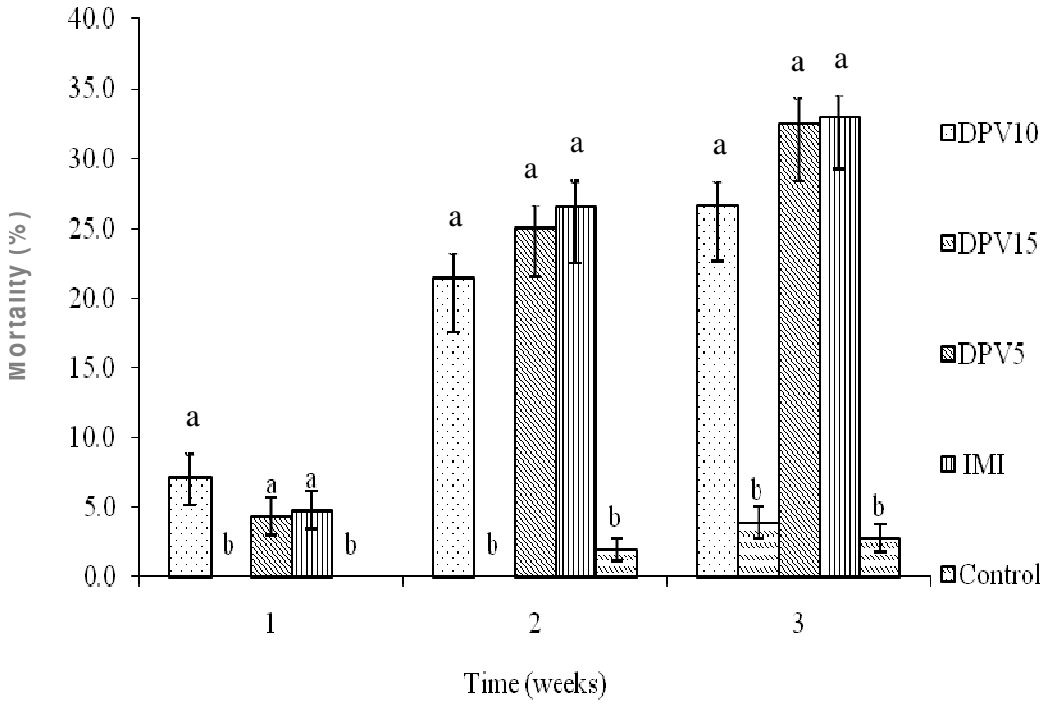

Figure 2. Cumulative mean mortality per week of *Z. variegatus* infected with 3 local strains of *M. anisopliae* DPV10, DPV15, DPV5 and IMI330189. Within a week means with same letters are not significantly different by Student Newman Keuls test.

significant differences among DPV5, DPV10 and IMI330189 in the first week. Similar trend of mortality followed in week 2 (Figure 2). DPV15 was not pathogenic to *Z. variegatus*. DPV5 and IMI330189 had the shortest LT_{50} (Table 2).

Comparison of the pathogenicity of the *M. anisopliae* strains on both *L. migratoria* and *Z. variegatus*.

L. migratoria was more susceptible to *M. anisopliae* than *Z. variegatus*. A t-test analysis showed that, there was significant difference of pathogenicity of the *Metarhizium* isolates on the two species ($P < 0.05$) (Table 3). In all the treatments, the overall mortality was more important on *L. migratoria* than on *Z. variegatus*.

DISCUSSION

According to Shapiro-Ilan et al. (2005), pathogenicity is the quality or state of being pathogenic, the potential ability to produce disease, whereas, virulence is the disease producing power of an organism and the degree of pathogenicity within a group or species (Shapiro-Ilan et al., 2005). In this study, there were varying level of pathogenicity. This can be explained by the fact that, the strains do not have the same origin; for instance IMI330189 has been isolated from Niger on *O. cavroisi* Finot, whereas DPV5, DPV10 and DPV15 were isolated from Senegal.

Table 3. Comparison of pathogenicity of 3 local strains of *M. anisopliae* DPV10, DPV15, DPV5 and IMI330189 on *L. migratoria* and *Z. variegatus*.

Strain	1 week		2 weeks		3 weeks	
	t -Value	p-Value	t -Value	p-Value	t -Value	p-Value
DPV10	4.3	<0.0001	7.8	<0.0001	10.0	<0.0001
DPV15	3.2	0.0022	4.6	<0.0001	5.9	<0.0001
DPV5	3.7	0.0004	10.2	<0.0001	11.0	<0.0001
IMI	3.5	0.0007	9.2	<0.0001	11.5	<0.0001
Control	.	.	3.2	0.0022	4.1	<0.0001

t-test value and p are level of significance.

Although, DPV15 and DPV10 were isolated from *O. cavroisi* (Bon et al., 2003), the differences in genetic patterns within the strains could explain the differences of pathogenicity (Bidochka et al., 2005). Insect pathogenic fungi such as *M. anisopliae* invade the host cuticle directly and enter the haemolymph. Following entry, the fungi must adapt to the host haemolymph environment before beginning to proliferate as a yeast-like phase (blastospores) (Seyoum et al., 2002; Zhang and Xia, 2009). In that regard, *M anisopliae* produces a wide range of enzymes during the process of infection. It has been reported that, different isolates of *Metarhizium* grown a media containing locust cuticles produce variety of extracellular enzymes corresponding to cuticles (St-Leger et al., 1986; St-Leger et al., 1996). This can explain the differences observed in our study: IMI330189, DPV5 and DPV15 were more pathogenic on *L. migratoria*. On the other hand, DPV15 was not pathogenic to *Z. Variegatus*. Our study has revealed that, the *M. anisopliae* var. *acridum* local strains are pathogenic to *L. migratoria* and *Z. variegatum*. In that particular species, previous works reported a possibility by some insects to lengthen the infection by developing a fever. For instance, *Z. variegatus* is a weak thermo regulator and is expected to be susceptible to *M. anisopliae* (Blanford et al., 2000; Elliot et al., 2002). However another strain, I91-609 is known to be highly pathogenic to *Z. variegatus* (Lomer et al., 1999). Wilson et al. (2002) reported that, locust reared in crowded conditions are more resistant than solitary stages (Wilson et al., 2002). Besides, the antifungal activity in the gregarious stages is higher than in solitary stages. During the cause of this study, we kept the insects in small boxes, there was more frequent moulting on *L. migratoria* larvae than on *Z. variegatus* after infection; however, over 90% cadavers were infected except in the control. Our results confirm the finding of Lomer et al. (2001). Highest mortalities of insects were recorded within 14 to 20 days. It has been reported that, grasshoppers can still be infected by the fungus up to three weeks after application (Thomas and Jenkins, 1997). DPV5 which has been isolated from Kaolack Kaffrine is a promising isolate for the control of locusts and local grasshoppers. Therefore, further investigations to determine the host range and its potential development for the control of locust and other local grasshoppers in Senegal is recommended.

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