African Journal of Agricultural Research Vol. 7(27), pp. 3876-3881, 17 July, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.541
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Susceptibility of *Callosobruchus maculatus* (Coleoptera: Bruchidae) to strains of *Metarhizium anisopliae*

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Accepted 16 February, 2012

The susceptibility of the bean beetle *Callosobruchus maculatus* to *Metarhizium anisopliae* var. *anisopliae* (URM3349) and *Metarhizium anisopliae* var. *acridum* (URM4412) strains was assessed at concentrations of 10^8, 10^7, 10^6, 10^5 and 10^4 conidia.ml^{-1} under laboratory conditions. The beetles were raised in glass recipients on a natural diet of cowpeas (*Vigna unguiculata*). Concentrations caused mortality in adult insects. At the concentration of 10^8 conidia ml^{-1}, mortality of *C. maculatus* caused by *M. anisopliae* var. *anisopliae* and *M. anisopliae* var. *acridum* was 74.45 and 58.27%, respectively. The LC_{50} of *M. anisopliae* var. *anisopliae* was estimated at 9.2×10^3 conidia ml-^{1}. The results demonstrated that the fungal strains evaluated have pathogenic action against the bean beetles under laboratory conditions.

Key words: Entomopathogenic fungus, *Metarhizium anisopliae*, pathogenicity, *Callosobruchus maculatus*, mortality.

INTRODUCTION

The bruchid, *Callosobruchus maculatus* (Fabr, 1976), is one of the main agents that attack cowpea, *Vigna unguiculata* (L.), and is an important pest that occurs in Northeastern Brazil, where a large portion of the beans cultivated belong to this genus. The cowpea is of considerable importance to Brazil as a widely used food source for both rural and urban populations due to its high protein content, accessible cost and greater nutritional content in comparison to common beans. Moreover, its leaves are used as a complement to animal feed (Andrade et al., 2005). The main damage caused by cowpea weevil to the grains resulted from the galleries made by the larvae causing weight loss, reduced nutritional value and decrease of germination of seeds. Furthermore, the presence of insects, eggs and excrement devaluate the grains commercially (Oliveira et al., 2000; Almeida et al., 2005; Melo et al., 2010). The control of the cowpea weevil was mainly carried out by means of chemical products. However, the inadequate use and the need for the reapplication of pesticides in increasing quantities raised the cost of this type of treatment and led to product loss due to inefficient control, as well as, the contamination of beans and farmers alike (Athié and De Paula, 2002). In view of these problems, entomopathogenic fungi are an alternative of proven efficacy in the control of different types of pests. These agents are safe for non target organisms, have longer lasting effects, remain in the environment for longer periods of time and

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Table 1. Means percentage mortality of *Callosobruchus maculatus* caused by different concentrations of *Metarhizium anisopliae* var. *anisopliae* and *Metarhizium anisopliae* var. *acridum* under laboratory conditions.

Strain	Concentrations (Conidia.ml^{-1})					Average
	10^4	10^5	10^6	10^7	10^8	
URM3349	33.63^{aC}	34.90^{aC}	57.72^{aB}	69.18^{aAB}	74.45^{aA}	53.98^{aA}
URM4412	34.81^{aC}	40.36^{aBC}	41.81^{bBC}	48.27^{bAB}	58.27^{bA}	44.70^{bB}
VC	23.75*					
	12.12**					

Means followed by the same letter, (upper case) in the row and (lower case) in the column, do not differ by Turkey's test at 5%. VC = variance coefficient: *, URM3349; **, URM4412.

can be used with selective insecticides, thereby, reducing the amount and number of applications of chemical pesticides (Vieira, 1988; Toriello et al., 2006). Based on these considerations and in view of the potential of this fungus to control stored product pests, the efficiency of *M. anisopliae* var. *anisopliae* (URM3349) and *M. anisopliae* var. *acridum* (URM4412) strains in the control of *C. maculatus* in matured adult phase under laboratory conditions was evaluated.

MATERIALS AND METHODS

Fungal strains

M. anisopliae var. *anisopliae* (PL43) originally isolated from *Mahanarva posticata* (Stal.) from the state of Alagoas (Brazil) and *M. anisopliae* var. *acridum* (CG291) originally isolated from *Austracnis guttulosa* (Walker) from Australia. The isolates were deposited at the Mycology Culture Collection, Micoteca URM of the Department of Mycology, Federal University of Pernambuco, Brazil, under accession numbers *M. anisopliae* var. *anisopliae* (URM3349) and *M. anisopliae* var. *acridum* (URM4412).

Callosobruchus maculatus

The test insects (*C. maculatus*) were raised from matrices obtained from the Agricultural Entomology Laboratory, Department of Agronomy, Federal Rural University of Pernambuco, Brazil. The insects were raised in recipients of transparent glass containing 0.5 kg of previously expurgated cowpeas, sealed with a thin voile cloth to allow aeration. The insects were confined for four days for egg laying and were later removed. The recipients were stored until the emergence of the adults. The *in vitro* analysis was performed with recently emerged adults (24 h) (Santos, 1976).

Pathogenicity test

The two isolates used bioassays and were placed on Petri dishes with PDA, and incubated under the room conditions (temperature (28 ± 2°C), 70 to 75% relative humidity, and 12-h photoperiod). The conidia were suspended in 0.05% Tween 80 solution (v/v) and shaken for 3 min to homogenize the solution, obtaining a final concentration of 1 × 10^8 conidia ml^{-1}. The adults isolated after emergence were sprayed with 0.5 ml of the fungal suspension using a manual mini-sprayer (Mainland, model nº XJ1^{-6}). Five concentrations (10^8, 10^7, 10^6, 10^5 and 10^4 conidia ml^{-1}) of each isolate were used with ten replicates of ten insects each. Control insects received the same volume of sterile water (0.05% Tween 80). Then numbers of dead live insects were counted once a day for twelve days. Dead insects were kept in a humidified hot house for the confirmation of death by the fungal agents (Paz Júnior, 2006).

Statistical analysis

The data on mortality were submitted to analysis of variance and comparison of means using Turkey's test. The Statistical and Genetic Analysis System program, version 5.0 (Euclides, 1985), was used. Probit analysis was used to determine LT50, of each isolate in the different treatments using the POLO PC program (LeOra Software, 1987).

RESULTS AND DISCUSSION

The efficiency of the *M. anisopliae* isolates was determined by the mean percentage of mortality, which ranged from 6 to 92.4% over the course of eleven days following inoculation. Although, there are reports in the literature stating that *C. maculatus* adults survived seven to nine days (Gallo et al., 2002), under laboratory conditions in the present experiment and a survival of greater than eleven days was observed. Cherry et al. (2005) reported a similar finding on the analysis of the daily mortality rate of insects immersed in an aqueous suspension with *M. anisopliae* for twelve days. There was a significant increase in mortality of the insect with increased concentration of the conidia in the suspensions. There were no significant differences between the two strains at concentrations of 10^4 and 10^5 conidia ml^{-1}. However, the strains differed significantly at the remaining concentrations with the URM3349 strain achieving higher percentages of insect mortality (Table 1).

Comparing the percentages of *C. maculatus* mortality, both stains of *M. anisoplie* proved pathogenic, but the URM4412 strain was less virulent, taking much longer to achieve a 50% mortality rate. The URM3349 was more pathogenic killing more than 50% by the fifth day following inoculation. Barreto et al. (2004) also used these

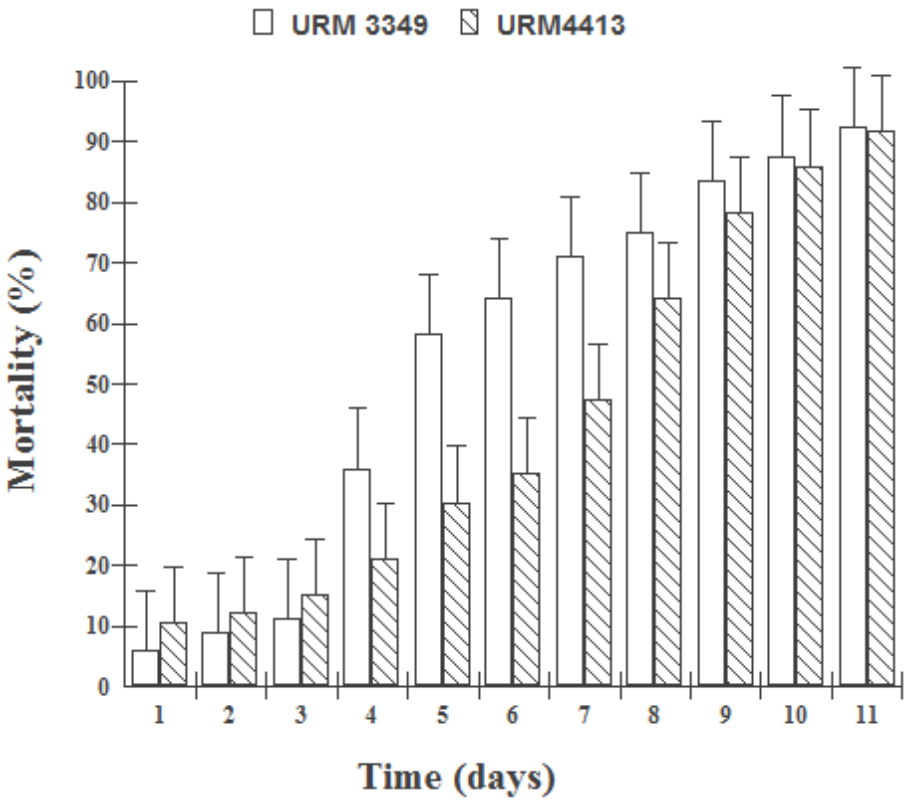

Figure 1. Comparison of the mortality among the strains of *Metarhizium anisopliae* var. *anisopliae* URM3349 and *Metarhizium anisopliae* var. *acridum* URM4412 under laboratory conditions.

parameters to identify the more pathogenic strain, with the URM4412 achieving greater than 50% mortality of the insects only beginning at the seventh day. In the present study, there were no statistically significant differences between the two strains from the ninth to twelfth days following inoculation (Figure 1). Thus, both strains proved virulent over time as represented by the cubic polynomial model (Figures 2 and 3).

Cherry et al. (2005) also assessed the pathogenicity of *M. anisopliae* over *C. maculatus* under laboratory conditions in immersion bioassays using the same concentrations as those in the present study, but employing a different application method. The authors reported non-corrected accumulated mortality percentages ranging from 29.99 to 83.33% on the sixth day. In the present study, mean mortality percentages on the sixth day ranged from 23 to 100% with the URM3349 isolate and 20 to 53% with the URM4412 isolate for the concentrations of 10^4 and 10^8 conidia ml^{-1}, respectively. With the URM3349 strain, the lethal time decreased with the increase in concentration, except at the lowest concentrations (10^4 and 10^5). At 10^4 conidia ml^{-1}, LT_{50} (time elapsed until the mortality of at least 50% of the individuals) was between the eighth and ninth day and maximal mortality (100%) was not achieved by the end of the experiment. At 10^5 conidia ml^{-1}, LT_{50} was also between the eighth and ninth day following inoculation. At 10^6 conidia ml^{-1}, greater than 50% mortality was achieved beginning on the fifth day. Using this same strain and concentration on *Nasutitermes coxipoensis* soldiers and workers, Albuquerque et al. (2005) report a mean LT_{50} on the first day following inoculation. However, variations in the defense mechanisms of these different arthropods may time lead to different responses to the same pathogenic agent. Due to the few references on the use of *M. anisopliae* for the control of the bean beetle, the effect of this entomopathogen on other arthropods is also considered.

Maximal mortality (100%) of the *C. maculatus* adults at concentrations of 10^7 and 10^8 conidia ml^{-1} occurred on the ninth and fifth day following inoculation. Loureiro and Monteiro (2005) reported on maximal mortality of soldier ants on the fourth day following inoculation. Mean LT_{50} in the present study was less than three days at concentrations of 10^7 and 10^8 conidia ml^{-1}. Reis et al. (2001) reported less than 65% mortality with the 959 isolate of *M. anisopliae* on *Amblyomma cajennense* adults. Figuerêdo et al. (2002) assessed the pathogenicity

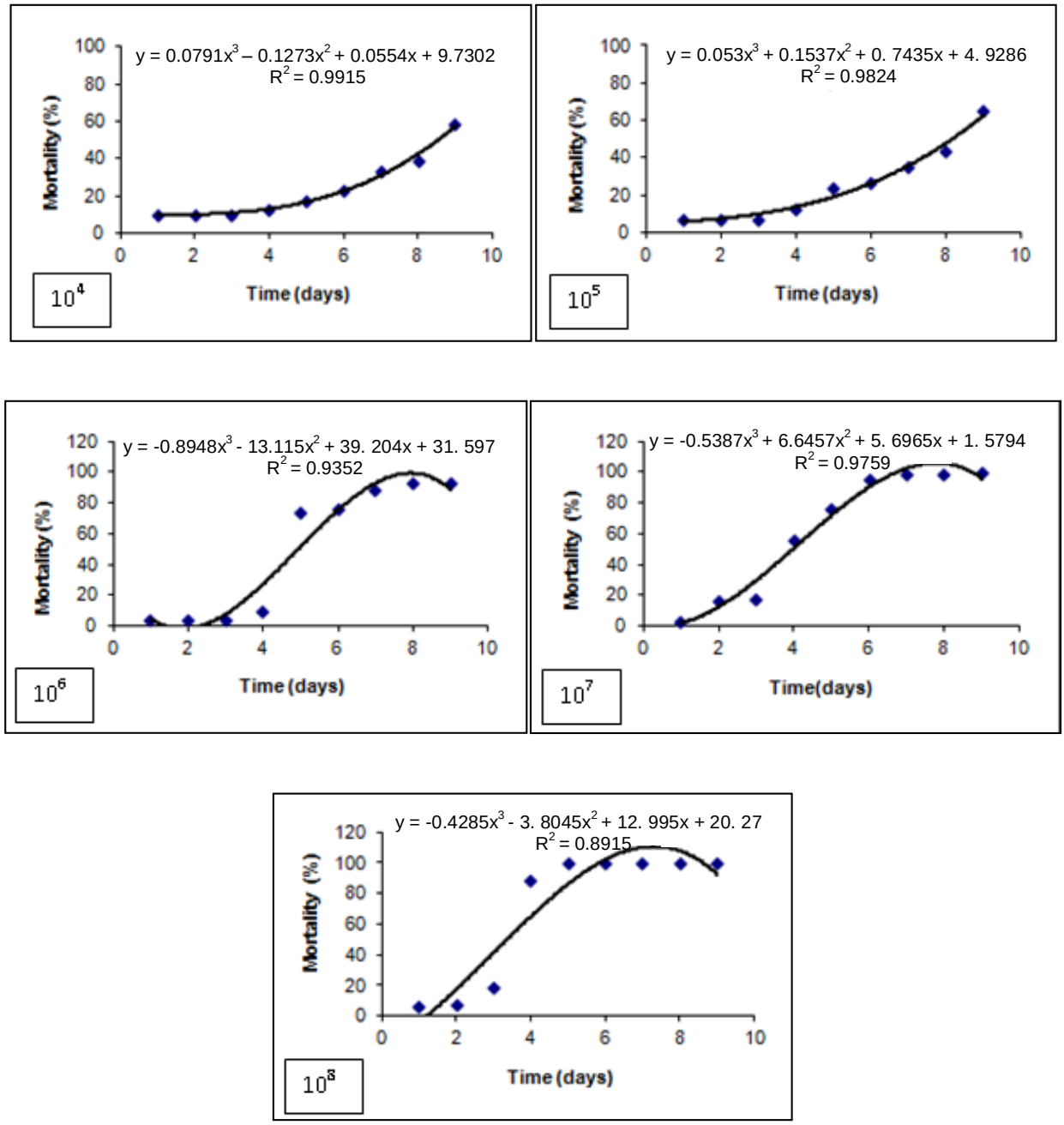

Figure 2. Means percentage of accumulated mortality of *Callosobruchus maculatus* caused by different concentrations of *Metarhizium anisopliae* var. *anisopliae* URM3349 under laboratory conditions.

of different *M. anisopliae* isolates over *Castnia licus* for which the lowest LT_{50} value was 7.3 days.

The results of the present study demonstrate a direct proportional relationship between the amount of conidia administered and the mortality rate of *C. maculatus*. This finding was also described by other authors studying isolates from the same fungus on different species of insects (Alves et al., 1985; Silva et al., 2003). According to Fernandes and Alves (1992), a greater number of conidia led to the release of more toxins or enzymes, thereby, increasing the mortality of the insect. However, the action velocity of the fungus also depends on the host species involved (Sosa-Gómez and Moscardi, 1992).

According to Leger et al. (1991), variation in the virulence of isolates of entomopathogenic fungi is related to the chemical concentration of the cuticle and the biochemical

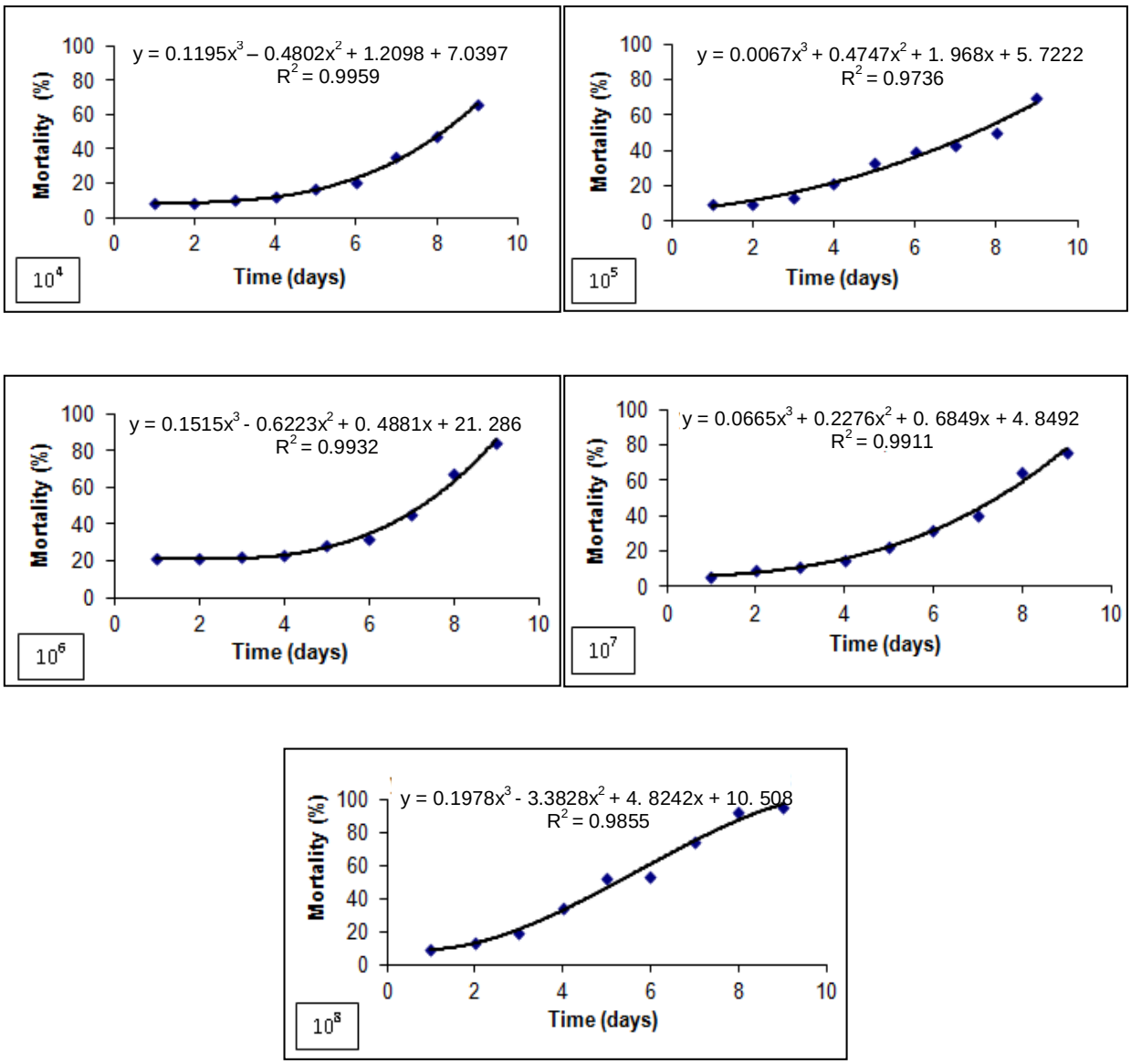

Figure 3. Means percentage of accumulated mortality of *Callosobruchus maculatus* caused by different concentrations of *Metarhizium anisopliae* var. *acridum* URM4412 under laboratory conditions.

processes involved in the formation of the germinative tube and colonization of the host. Moreover, Oliveira et al. (2004) stressed that the capacity of fungus to cause death stems is from the ability of its conidia to recognize and produce enzymes that breakdowns the host cuticle.

In conclusion, there was a significant increase in the percentage of mortality of the bean beetle with the increase in the concentration of the fungus (Figure 3). The URM4412 isolate caused *C. maculatus* mortality at all concentrations tested, with mean values ranging from 34.82 to 58.27%. The *M. anisopliae* URM3349 isolate was more pathogenic to *C. maculatus* based on LT_{50} and LC_{50} values. However, the data obtained for the URM4412 isolate did not adjust to the Probit model, probably because the bioassays did not always follow the stimulus-response model (Haddad, 1998). In the Probit analysis, the strains tested were not suitable for the model, as there was a significant χ^2 and considerable heterogeneity of the data. In experiments with micro-organisms, a linear model is not always seen in the stimulus-response relationship, which hinders the analysis using this model (Paz Júnior, 2006). Thus, the data presented here demonstrated that strains of *Metarhizium anisopliae* can infect *C. maculatus*, but their efficiency varies depending on the origin of the isolate. Despite the efficacy of the strains tested here, there is a

need for further studies to assess their potential in the field before using them in biological pest control programs.

ACKNOWLEDGMENTS

The authors are grateful to CENARGEN-EMBRAPA (Biological Control Sector) for the *M. anisopliae* var. *acridum* CG291 strain and the Brazilian fostering agency Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

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