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Solid Substrate Production and Formulation of an Isolate of *Metarhizium anisopliae* for Biological Control of Stem Bug *Tibraca limbativentris*

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Abstract: One isolate of *Metarhizium anisopliae* (Ma 72) previously selected for its virulence against rice-stem bugs, *Tibraca limbativentris,* was studied. Its conidial production and quality parameters in several solid substrates, as well as its reusability, were evaluated. Conidial germination percentages (%G) in two experimental formulations - broken white rice and parboil rice - were evaluated at different storage times (0, 50 and 100 days) at 6°C and 20°C. Ma 72 was able to produce conidia in all the substrates. When used for the first time, the broken white rice presented the highest production parameters, with 4.4 $x10^{10}$ conidia/g conidial powder. Its production parameter was even higher than that of parboil rice (control). Nevertheless, when the substrates were reused, parboil rice presented the highest value. In both experimental formulations, the%G decreased from 97% to less than 87.32% and 85.11% at 6°C after 50 and 100 days of room storage, respectively. At 20°C the%G presented values below 51.42% and 16.47% after 50 and 100 days of storage, respectively. Only parboil rice could be reused successfully, but at a considerable cost. In contrast, broken white rice might be suitable as an economical substrate but would not be reused, thus probably increasing production wastes. Using broken white rice as part of a wettable powder formulation may reduce these wastes.

Key words: Solid Substrate Fermentation · Rice · Entomopathogenic Fungi · Fungal Formulation · Germination

INTRODUCTION

Rice farming is one of the main productive activities in the Northeast of Argentina, where different pests reduce its yield and quality. It represents an important income to the country, arising not only from national intake but also from exports [1]. Rice farming is affected by different pests that reduce rice yield and quality. One of the pests affecting rice farming is the stem bug, *Tibraca limbativentris* Stal, 1860 (Hemiptera: Pentatomidae), which is distributed throughout the rice-growing region of Argentina [2], causing serious problems for rice farmers. This pest damages the rice crop (*Oryza sativa*) from the beginning of the tillering. In paddy fields, the insects are located at the base of the plants, among their stalks, preferably where there is no formation of an irrigation sheet and the soil is saturated [3]. In these places, micrometeorological conditions of humidity and temperature result not only in the growth of the insect population [4], but in the development of entomopathogenic fungi as well [5].

Entomopathogenic fungi are ideal candidates for integrated pest management (IPM) in forests and greenhouses [6] and controlling stem bugs requires the development of an IPM programme. An interesting candidate for such a programme is *Metarhizium anisopliae* (Metchnikoff) Sorokin because it is widely distributed in nature, infecting over 200 species of insects and acari [7]. Its active unit, the fungal conidia, may be massively produced by employing different mediums. Fungal conidia have been produced on the surface of solid mediums since the 1970s. Their current production methods have been developed and improved by Alves and Pereira [8]. Several solid substrates, such as rice,

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bean, sorghum, wheat and millet flours, have been used for this purpose. Vilas Boas *et al*. [9] found that rice has achieved the best substrate to produce the related fungus, *Beauveria bassiana.* However, to the best of our knowledge, no descriptions of the kind of rice that achieved the best relation cost-production of *Metarhizium* spp conidia have been done.

Production technologies have been developed for aerial conidia and submerged spores of entomopathogenic fungi [10- 12]. A promising method to produce spores of *M. anisopliae* is solid state fermentation (SSF). The aerial conidia produced by means of SSF are similar to those produced naturally on the surface of insect cadavers and are superior to mycelia and blastospores produced under liquid culture fermentation (LCF) [13-15]. Mass production directly influences the cost, shelf life, virulence and field efficacy of fungal products. Numerous studies have shown that nutritional and environmental conditions during fungal growth using SSF and LCF influence the form and efficacy of the fungal conidia [16-18]. For instance, the nutritional composition of the production medium has a significant impact on the attributes of the resulting conidia, such as biocontrol efficacy, desiccation tolerance and persistence [19].

From the standpoint of biotechnology, a variety of fungal conidia can be produced using SSF. For biological control, it is important to keep the active unit, for instance fungal conidia, in an infectious yet dormant stage, which is safe and easy to apply. An optimum formulation must ensure the biological and chemical stability of the product in room storage, as well as the viability and biocontrol activity of the microorganism [20]. Formulations can be employed in order to alter the chemical and physical attributes of fungal conidia to improve their insecticidal activity under varied environmental condition [21]. In addition, formulation of the fungal conidia or the use of adjuvants during application can also influence efficacy [22-24].

It has been demonstrated that some oils present not only a good performance as adjuvants in formulations in the mycoinsecticides development [25-28], but also a synergistic effect on the control of plagues [29-32].

More than 150 insect biocontrol products based on entomopathogenic fungi have been commercialized with over 75% of these products based on the Hypocrealean fungi *M. anisopliae*, *B. bassiana*, *B. brongniartii* and *Isaria fumosorosea* [33]. However, no mycoinsecticide has been registered in Argentina yet.

With the purpose of developing a mycoinsecticide to be used in integrated management programmes of *T. limbativentris*, we investigated the effectiveness of several substrates in the conidial production and quality parameters of one isolate of *M. anisopliae,* previously selected for its virulence against *T. limbativentris.* We also evaluated the performance in room storage of two kinds of formulations based on this isolate.

MATERIALS AND METHODS

Fungal Isolate and Culture: One *M. anisopliae* isolate (Ma 72), previously selected for its virulence against *T. limbativentris,* was used in this research. It was isolated from *T. limbativentris* and deposited at the collection of the Laboratory of Entomopathogenic Fungi (LEF) of the Institute of Microbiology and Agricultural Zoology (IMyZA) of the National Institute of Agricultural Technology (INTA), Argentina. The isolate Ma 72 was cultured on Potato Dextrose Agar (PDA; Oxoid) and incubated at 25°C for 7-10 days.

Evaluation of Conidial Production and Quality Parameters in Several Solid Substrates: The substrates used were parboil rice (Control), whole white rice, broken white rice, paddy rice, brown rice, rice husk and husked wheat.

The fungus was cultivated in 20 x 40 cm polypropylene bags. Three replicates were done for each substrate. An aliquot of 45 g of substrate was placed in each bag and 22.5 mL of distilled water was added in order to reach 45% relative humidity (RH) in all substrates. The bags were sealed, autoclaved for 20 min at 121°C and then inoculated. The inocula were produced on potato dextrose agar (PDA, Oxoid) amended with chloramphenicol (0.5 $g.L^{-1}$) and 100 µl of a 1 x 10^7 conidia/ml suspension was used to inoculate each bag in a laminar flow chamber (ESCO AC2 - 4A1). After inoculation, the bags were homogenized and incubated at 27±1°C and 16:8 photoperiod for 12 days. After fungal growth, the bags were opened and the substrate was dried in darkness using two mobile dehumidifier (Dryclim 27 Model Carel) until RH < 10% for 48 h. Conidia were sieved by an electronic vibrator Zonitest LR 2006 (2500 rpm) during 20 min using a metallic mesh (300 µm). Conidial powder was collected in metallic bags inside a laminar flow chamber and the weight was registered. When we referred to conidial powder above, we meant not only the conidia of the fungus, but also the remaining rice particles that mingled with conidia during the sieving process.

The following parameters were calculated from the conidial powder obtained from each treatment (Substrate) and the control:

Productivity Parameters:

- Grams of conidial powder produced per kg of substrate: To obtain this parameter, both conidial powder and the substrate were expressed in dry matter using a stove at 80-90°C until constant weight of the powder and the substrate respectively.
- Number of conidia per g of conidial powder: An improved Neubauer chamber and an optical microscope were used.
- Number of conidia per kg of substrate: It was obtained by multiplying the grams of conidial powder produced per Kg of substrate * Number of conidia per g of conidial powder.

Quality Parameter:

- Conidial germination percentage:

A small portion of conidial powder (0.15 -0.2 mg) was raised on the tip of a spatula and transferred to a tube with 10 ml of Tween 80 (0.05%). Suspension was placed on a glass Petri plate containing PDA + 0.5% (w/v) Chloramphenicol + 0.002% (w/v) Benomyl with 25% active ingredient (Punch Química SA, Argentina). Benomyl has little effect on germination, but severely inhibits the growth of germ tubes, thereby preventing overgrowth of mycelium and allowing germination to be monitored for up to 24 h [34]. Three replicates were done for each treatment. The plates were incubated in at 25±1°C. After 24 and 48 h, one drop of lactophenol cotton blue and a coverslip were placed on the plates and germination was observed at 400 x magnification in an optical microscope. For each plate, four microscopic fields, each containing a minimum of 100 conidia, were evaluated.

Remaining Substrate Parameters:

- Percentage of remaining substrate: It was calculated by multiplying dry weight of the remaining substrate *100 / initial weight of the remaining substrate.
- Remaining substrate humidity: It was obtained using a stove at 80-90°C until constant weight and the following formula was applied: Remaining substrate humidity (%) = 100 - (Dry weight remaining substrate *100 / initial weight of the remaining substrate)
- Substrate utilization efficiency of the fungus: It was calculated using the following formulae:

% substrate utilization efficiency = g conidia produced per kg substrate x 100 / (Kg used substrate – kg remaining substrate).

Data were analyzed using the statistical package R version 2.12.0. Production parameters from each treatment were compared using ANOVA and a Tuckey test ($\alpha = 0.05$) was performed for post-hoc comparison.

Effectiveness of the Reused Substrate: To evaluate the effectiveness of the substrate already used once to produce the strain Ma 72, the substrates that presented the best results in the production and quality parameters were selected. These substrates were whole white rice, broken white rice, brown rice and parboil rice (Control).

The evaluation of the parameters was performed as described in subsection 2. Data were analyzed using the statistical package R version 2.12.0. Production parameters from each treatment were compared using ANOVA and a Tuckey test ($\alpha = 0.05$) was performed for post-hoc comparison. In addition, values of germination percentage at 48 h of incubation were compared with the same percentages obtained from the first time the substrate was used and were analyzed by a T test.

Selection of Formulation Carriers: A suspension of Ma 72 was prepared and adjusted to 1 x 10^7 conidia / ml using a Neubauer improved chamber. Five formulation carriers were evaluated. The formulation with Tween 80® was the control of the experiment. Carriers were added to the suspension as shown in Table 1. Two replicate were performed for each treatment. Each suspension was shaken during 2 h at room temperature. From each treatment, germination percentage was performed as described in subsection 2.

The experimental design was randomized. Data were analyzed using the statistical package R version 2.12.0. Germination values from each treatment were compared using ANOVA and a Tuckey test ($\alpha = 0.05$) was performed for post-hoc comparison.

Experimental Formulations from Ma 72: Two kinds of experimental formulations from Ma 72 isolate were evaluated: wettable powder and oil emulsifiable concentrate (Table 2). Both experimental formulations were prepared with 15% of active ingredient in a 15 ml test tube and sealed with cotton plug. Each test tube was closed using a pair of polypropylene bags to control formulation moisture. Control was prepared with 100% dry conidia. Two replicates were done for each experimental formulation.

Table 1: Formulation aids and concentrations evaluated

Formulation aid	Trademark	Class	Concentration
Polysorbate ester	Tween 80®	Non-ionic surfactant and emulsifier	5% (v/v)
Linear fatty alcohol alkoxylate	Plurafac®	Adjuvant, moisturizer, Non- ionic adherent	5% (v/v)
			10% (v/v)
Minarel oil, saturated hydrocarbon mixture	Bayer Xtra®	Emulsifiable adjuvant	5% (v/v)
			10% (v/v)
pH corrector	Total I®	Emulsifiable adjuvant	1.5% (v/v)
Soy vegetable oil	Oleo®	Non-ionic adjuvant	5% (v/v)

Table 2: Composition of the formulations of Ma 72 stored at two temperatures

Term Storage	Formulation types	Active ingredient	Inert
6 °C	Wet powder (WP)	100% Dry spore powder	-
		15% Dry spore powder	85% Corn starch
			85% Talc mineral
			85% Ground rice
	Emulsifiable Concentrate (EC)	15% Dry spore powder	85% Oleo®
			80% Oleo® + 5% Plurafac®
			80% Oleo® + 5% Bayer Xtra®
			80% Oleo® + 5% Tween 80®
20° C	Wet powder (WP)	100% Dry spore powder	-
		15% Dry spore powder	85% Corn starch
			85% Talc mineral
			85% Ground rice
	Emulsifiable Concentrate (EC)	15% Dry spore powder	85% Oleo®
			80% Oleo® + 5% Plurafac®
			80% Oleo® + 5% Bayer Xtra®
			80% Oleo® + 5% Tween 80®

Germination Capability of Experimental Formulation in Room Storage: The experimental formulations were maintained at two room storage temperatures: 6 and 20°C. The germination capability of each formulation was evaluated at the beginning of the experiment and at 50 and 100 days after room storage. Conidial germination was evaluated as described above in subsection 2.

This experiment presented a factorial design with two factors (Formulations with 8 levels and temperature with 2 levels) and two replicates. Data were analyzed by means of a three-way ANOVA using the statistical package R version 2.12.0. Germination values from each treatment were compared using ANOVA and a Tuckey test ($\alpha = 0.05$) for post-hoc comparison.

RESULTS AND DISCUSSION

Evaluation of Several Solid Substrates in Parameters of Conidial Productivity and Quality: Productivity and quality parameters obtained from the isolate Ma 72 using several solid substrates are shown in Table 3. All substrates showed significant differences in the productivity parameters. Isolate Ma 72 was able to produce conidia in all the substrates evaluated. The broken white rice presented the highest production parameters with 3.72×10^9 conidia/g substrate ($P < 0.05$), 85.52 g conidial powder/ kg substrate ($P < 0.05$) and 4.4×10^{10} conidia/ g conidial powder ($P < 0.05$). Its production parameters were even higher than t hose of the parboil

Table 3: Productivity parameters of conidia of Ma 72 produced on several solid substrates

Solid substrate	Conidial powder (g / kg substrate)	Number conidia/ g conidial powder	Number conidia/g substrate	Germination percentage (%)
Broken white rice	85.52 A	4.40 x 10^{10} A	3.72x 10^{9} A	98.31 A
Parboil rice (control)	57,85 B	3,63 x 10^{10} BC	2.04 x 10^{9} A	97.98 A
Whole white rice	44.39 BC	3.33 x 10^{10} BC	1.44 x 10^{9} BC	97.92 A
Brown rice	43.07 C	2.07 x 10^{10} CD	8.9 x 10^{8} CD	97.69 A
Paddy rice	16.29 D	3.7 x 10^{10} BC	6.04 x 10^{8} CD	97.84 A
Husked wheat	8.63 D	1.07 x 10^{10} D	9.22 x 10^{7} D	95.03 B
Rice husk	9.14 D	2.33 x 10^{9} D	2.11 x 10^{7} D	90.55 C

Means followed by the same letter within the same column, not significantly different by Tukey ($P < 0.05$).

Table 4: Remaining substrate parameters used for the production of Ma 72 conidia of *Metarhizium anisopliae*.

	Remaining substrate parameters		
Solid-Medium	Percentage of remaining substrate (%)	Remaining substrate humidity (%)	% Substrate utilization efficiency
Broken white rice	71,00 E	9,61 C	29,86 A
Parboil rice (control)	73,67 D	11,77 B	22,19 B
Brown rice	78,33 C	9,18 C	20,19 B
Whole white rice	77,00 C	9,14 C	19,23 B
Paddy rice	83,67 B	8,46 D	9,94 C
Rice husk	88,67 A	7,99 D	8,14 C
Husked wheat	60,00 F	36,22 A	2,17 D
CV (%	*1,03*	*1,41*	*8,89*
p-valor	*<0,0001*	*<0,0001*	*<0,0001*

Means followed by the same letter within the same column, not significantly different by Tukey ($P < 0.05$).

rice, which were used as control. The substrates that presented the highest production of conidia per gram were broken white rice (3.72 x 10^{9}), parboil rice (2.09 x 10^{9}) and whole white rice (1.44 x 10^{9}), in agreement with other authors´ results. For example, Rezende *et al*. [35] found that the same three substrates produced a similar number of conidia per gram (4.38 x 10^{9}, 2.68 x 10^{9} and 1.53 x 10^{9}, respectively). Ottati-de-Lima [36] concluded that parboil rice was the best substrate for conidial production of *M. anisopliae*. According to these results, several authors [9, 37, 38] concluded that parboil rice and whole white rice achieved the best substrates to produce conidia of *B. bassiana* and *M. anisopliae*.

The lowest production parameter values were observed on husked wheat, paddy rice and rice husk substrates. The values of conidial germination percentage decreased significantly ($P < 0.05$) when the substrates rice husk (90.55%) and husked wheat (95.03%) were used. Generally, substrates based on rice (With the exception of rice husk) presented values of conidial germination percentage higher than 97.5% (Table 3). Parameters obtained from the remaining substrate are shown in Table 4. Significant differences among the various substrates were observed in the values of remaining substrate percentage ($P < 0.05$). When comparing the utilization efficiency percentages of the substrates based on rice and those based on wheat, we found that the isolate Ma 72 made the most of the substrates based on rice. Results showed that the most efficient substrates for the fungal production were broken white rice, parboil rice, brown rice and whole white rice.

Rice is the most widely utilized substrate to produce conidia of entomopathogenic fungi. When produced in a non-technological way, these fungi have reached yields of 10^{9} to 10^{10} conidia per gram of substrate [39, 40]. These yields are similar to those obtained in this investigation.

Effectiveness of the Reused Substrate: Production and quality parameters of the reused substrate are shown in Table 5. The highest amounts of conidial powder were found in broken white rice (89.33%) and parboil rice (87.56%). The number of conidia per gram in conidial powder presented the highest value in parboil rice (2.39 x 10^{10} conidia/g conidial powder). The broken white rice produced the lowest number of conidia (2.56 x 10^{8} conidia/ g conidial powder). When we compared this parameter with that obtained in the first use of the substrate (Table 3), we observed a reduction in the values in the reused substrate with the exception of parboil rice, which did not vary markedly. Only parboil rice presented values similar to those found when the substrate was first used. Neves *et al*. [41] demonstrated that the reused rice presents a production 70% lower than that obtained when

Table 5: Parameters of conidia of Ma 72 produced in the reused substrate

Solid-Medium	Conidial powder (g / kg substrate)	Number conidia/ g conidial powder	Number conidia/g substrate	Germination percentage (%)
Broken white rice	89,33	2,56E+08	2,29E+07	98,22 A
Parboil rice (control)	87,56	2,39E+10	2,09E+09	95,09 A B
Whole white rice	73,62	2,74E+09	2,02E+08	78,17 C
Brown rice	45,56	5,77E+09	2,63E+08	91,44 B
CV (%)	-	-	-	*2,38*
p-valor	-	-	-	*< 0,0001*

Means followed by the same letter within the same column, not significantly different by Tukey (P < 0.05).

Table 6: Germination percentage of conidia of Ma 72 mixed with several formulation aids

Formulation aids	Germination percentage at 24 h (%)	Germination percentage at 48 h (%)
Control (Tween® 5% v/v)	86,34 A	85,05 A B
Plurafac® 5% v/v (Basf)	83,36 A	89,79 A
Plurafac® 10% v/v (Basf)	85,50 A	90,02 A
Bayer Xtra® 5% v/v	86,02 A	83,66 B C
Bayer Xtra® 10% v/v	84,63 A	84,06 B C
Total I Corrector® 1,5% v/v	77,62 B	78,83 C
Oleo® 5% v/v	81,73 A B	84,35 B
CV (%)	*2,51*	*2,61*
p-valor	*0,0002*	*< 0,0001*

Means followed by the same letter within the same column, not significantly different by Tukey (P < 0.05)

first used. While in the reused substrate we obtained a higher amount of conidial powder than in the first use of the substrate, the number of conidia per gram of conidial powder was lower than in the first use. One explanation for this would be the presence of a large number of residues in the reused substrate.

The highest values of conidial germination percentage were observed in broken white rice (98.22%) and parboil rice (95.09%). When the conidial germination percentage obtained from the first use of the substrates was compared with the same parameter obtained from the reused substrates, the values decreased ($P < 0.01$) with the exception of the broken white rice ($P = 0.78$).

Implementing economical substrates and reutilizing the substrate might be interesting tactics for reducing costs and production wastes, respectively. Nevertheless, in our study, we demonstrated that the only substrate that might be reused with profitable results was parboil rice, which is not the most economical substrate evaluated. In contrast, our findings showed that the broken white rice might be suitable as an economical substrate but would not be able to be reused and therefore, might increase production wastes. A profitable option to take advantage of the broken white rice already used and also to reduce the production wastes is to incorporate it in a formulation as its inert material. In this research, dried and ground broken white rice was used as inert material for the formulation WP: 15%Ma+85%ground rice. This formulation reached values of%G 83.02 at 6°C after 100 days of storage.

Selection of Formulation Carriers: The values of conidial germination percentage of Ma 72 mixed with the various formulation carriers are shown in Table 6. Non-significant differences among the percentages of germination of the mixtures were observed, as compared with the control (Tween 80® 5% v/v), except for the formulation carrier Total I Corrector®, which caused a significant decrease in the values of conidial germination percentage at 24 h and 48 h of incubation ($P < 0.05$). The mixture with both concentrations of Plurafac® presented higher values of conidial germination than the mixture with the carriers Bayer Xtra® and Oleo® at 48 h of incubation ($P < 0.05$). However, Bayer Xtra® and Oleo® behaved in ways similar to Tween 80® (control), so they could be considered in formulations. Kassa [42] demonstrated that Plurafac® did not affect the viability of conidia of one isolate of *M. anisopliae* and the authors concluded that this carrier resulted in a profitable formulation. In addition, Tween 80® has been widely used in laboratory assays to aid in the suspension of hydrophobic conidia in water [43-45]. Aerial conidia of *Metarhizium* sp. and *Beauveria* sp. are highly hydrophobic due to glycoproteins arranged in overlapping rodlets on the conidial surface [46]. This property makes oil carriers ideal for these conidia. All carriers evaluated were capable of forming a stable emulsion of oil in water, thus providing a basis for dispersing the active ingredient in suspension, specifically in order to formulate the isolate Ma 72. Also, they are soluble in water, so they may be successfully used in conventional hydraulic sprays in rice crop.

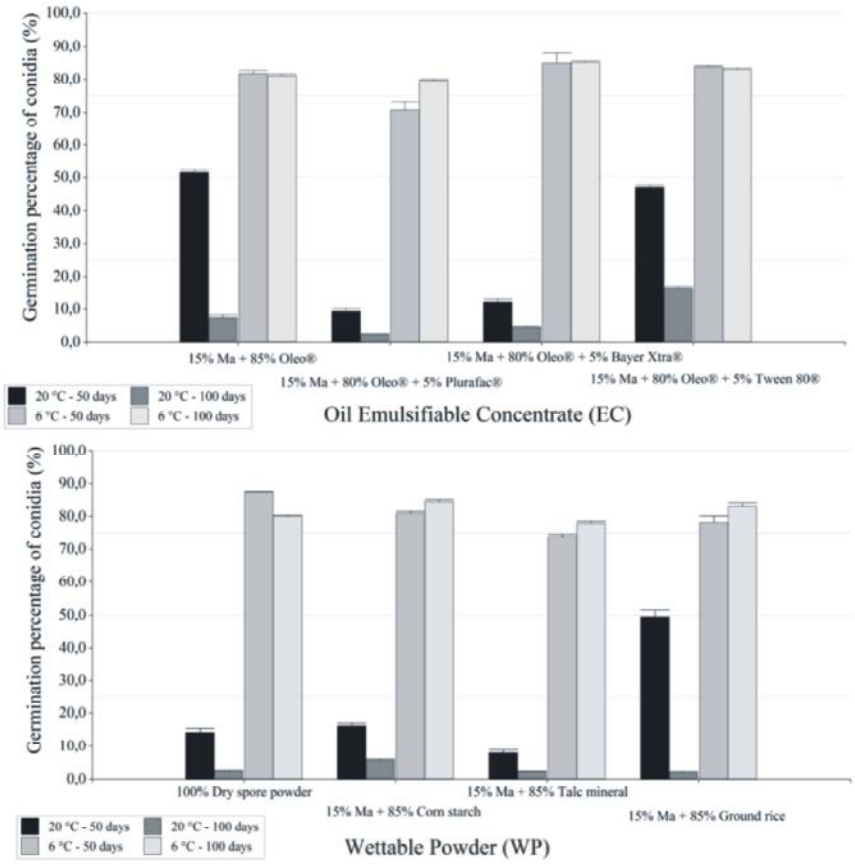

Fig. 1: Germination percentage Ma 72 conidia of *Metarhizium anisopliae* of the different formulations based Wet Powder (WP) and Emulsifiable Concentrate (EC) at different storage temperatures, 24 hours after incubation at 25 ± 0.5°C. Percentage of initial germination: 97%.

Germination Capability of Experimental Formulations from Ma 72 in Room Storage: Results of germination capability of several Ma 72 formulations in room storage are shown in Figure 1. The values of conidial germination percentage presented significant differences among the various formulations ($P < 0.05$), the room storage temperatures ($P < 0.05$) and the duration of the storage time ($P < 0.05$). In general, at 6°C, the values of conidial germination percentage decreased from 97% to less than 87.32% and 85.11% after 50 and 100 days of room storage, respectively. The lowest values observed at this storage temperature were 70.76% and 77.77% after 50 and 100 days, respectively. At 20°C the values of conidial germination percentage presented a greater decrease than those observed at 6°C, with values below 51.42% and 16.47% after 50 and 100 days of storage, respectively and minimum values of 7.82% and 1.96% after 50 and 100 days, respectively. In summary, conidial germination of isolate Ma 72 in 50 days achieved a better performance at 6°C than at 20°C for all the formulations evaluated. In addition, more significant differences were found among formulations stored at 20°C than among those stored at 6°C (Refrigeration temperature). After 100 days of storage, the formulations that had been kept at 20°C (Environmental temperature) drastically decreased their germination capability and very low values of conidial germination percentage were found (Lower than 16.47%). Nevertheless, in the formulations that had been kept at 6°C (Refrigeration temperature), the values of conidial germination percentage remained similar to those found after only 50 days of storage. At 6°C, the emulsifiable concentrates: 15%Ma+80%Oleo®+5%Bayer Xtra® and 15%Ma+80%Oleo®+5%Tween 80®, kept the conidial germination capability with values higher than 82% until 100 days of storage. Although Plurafac® was the carrier that presented the best performance, at 6°C the emulsifiable concentrate 15%Ma+80%Oleo®+5%Plurafac® was the worst formulation for keeping the germination capability settled for 50 and 100 days of storage.

The production and formulation goals for fungal entomopathogens must consider economic realities but also be mindful of the ecological constraint

requirements for consistent insect infection and control. New approaches to the production of efficacious fungal conidia and the development of formulations will lead to the availability of mycoinsecticides for controlling insects [47].

CONCLUSION

In conclusion, to produce Ma 72 successfully, the substrates based on rice, in particular broken white rice and parboil rice, should be used as they had the best performance. This work demonstrates that oil formulations of Ma 72 conidia, using Tween 80® and Bayer Xtra® and for a medium-term storage at refrigeration temperature (6°C) resulted in a successful formulation to be incorporated in Integrated Pest Management to control *T. limbativentris* in rice crops.

In this research, we only investigated the production and formulation of isolate Ma 72. However, further assays are needed to evaluate the performance of the formulations against the stem bug under field and laboratory conditions.

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