International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 3, 2019, pp.-1509-1513.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

NIGER SEED AGAR AS AN INDUCTOR OF SPORULATION OF FILAMENTOUS FUNGI WITH POTENTIAL IN BIOLOGICAL CONTROL OF AGRICULTURAL DISEASES AND PESTS

ITO F.A.D., PIMENTEL I. C. AND POITEVIN C.G.*
Laboratory of Microbiology and Molecular Biology (LabMicro), Department of Basic Pathology, Sector of Biological Sciences, Federal University of Paraná, 81530-900, Curitiba, Brazil
*Corresponding Author: Email - carol.poitevin@gmail.com

Received: February 23, 2019; Revised: March 25, 2019; Accepted: March 26, 2019; Published: March 30, 2019

Abstract- The culture media Niger Seed Agar, Sabouraud Malt Yeast Extract Agar, Oat Agar, Rice Agar and Sabouraud were evaluated for the vegetative growth and sporulation of the entomopathogenic fungi *Beauveria caledonica, Beauveria bassiana, Isaria javanica, Metarhizium anisopliae* and the endophytic fungi *Colletotrichum gloeosporioides, Botryosphaeria dothidea* and *Nectria pseudotrichia*, all with potential against pests and diseases. The Niger Seed Agar presented a significant difference regarding sporulation in relation to the other media and its production cost was lower comparing to SMAY. Therefore, the Niger Seed Agar medium was selected for optimization of temperature and photophase. In general, the factorial analysis showed that these factors influenced significantly the growth of endophytic fungi and the sporulation of entomopathogens. This is the first study to demonstrate the efficiency of the Niger Seed Agar for sporulation of filamentous fungi. It is an economically viable alternative for the development of processes aiming the commercial production of bioinsecticides and biofungicides.

Keywords- *Biocontrol, Culture medium, Photophase, Temperature*

Citation: Ito F.A.D., *et al.*, (2019) Niger Seed Agar as an Inductor of Sporulation of Filamentous Fungi with Potential in Biological Control of Agricultural Diseases and Pests. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 11, Issue 3, pp.-1509-1513.

Academic Editor / Reviewer: Deepa P M, Dr Vinod Kumar Singh, Dr Poonam Choudhary, Raghu B.R., Manjot Kaur

Introduction

The increase of extensive agriculture in the last decades has caused massive use of chemicals for the control of pathogens and pest insects, which negatively affects the quality of the food and the health of farmers and consumers. In addition, they may have harmful effects on non-target organisms, including beneficial plant insects, causing biological and ecological imbalance [1]. The use of endophytic and entomopathogenic microorganisms as biocontrol agents (BCA) has become a viable option, once those organisms are presented as alternatives that mitigate innumerable problems caused by the use of chemicals [2,3]. Endophytes exhibit the ability to penetrate the plant and to systematically disseminate in the host the same way phytopathogens do, however without causing any disease [4, 5]. They have been considered as an important and innovative resource of natural bioactive products with their potential applications in agriculture, medicine and food industry, attracting the attention of many researchers [6-8]. Species of entomopathogenic microorganisms are common in the Brazilian territory [9]. Once isolated and selected, they become an alternative for the production of bioinsecticides which have advantages such as low toxicity, specificity, easy dispersion and production. In addition, they enable the associated use with other methods of control, use of genetic engineering and the application of similar technologies to that used for chemical pesticides, but with a longer-term control that avoids imbalances and resurgence of pests with relatively low development and registration costs [10,11]. The success of a biological formulation relies on the viability of production at competitive costs, which means the production must have low cost and high yield, maintaining the efficiency of the BCA [12]. Making these products feasible involves the optimization of their production to make their large-scale use combined with integrated management possible [13]. The initial steps for large-scale production include determining the nutritional and physical requirements needed for the best growth [14, 15]. Therefore, the aim of this work was to evaluate the influence of physical and nutritional conditions on the growth and sporulation of endophytic and entomopathogenic fungi with potential in the biocontrol of diseases and agricultural pests.

Material and Methods

Fungal isolates

The isolates used are deposited at the Paraná State Microbiological Collection Network –TAXONline (CMRP), Department of Basic Pathology, Federal University of Paraná, Curitiba, Brazil [Table-1].

Inoculum preparation

The inoculum was carried out from spore suspensions at the concentration of 10^6 spores. mL^{-1} which were inoculated in Petri dishes containing Sabouraud Dextrose Agar (SDA-40 $g.L^{-1}$ dextrose, 10 $g.L^{-1}$ peptone, 15 $g.L^{-1}$ agar) and incubated for 4 days in a Biochemical Oxygen Demand (B.O.D.) chambers at 28°C ± 0.5°C.

Effect of substrate on vegetative growth and sporulation

Five culture media were evaluated: Rice Agar (RA-25 $g.L^{-1}$ rice, 18 $g.L^{-1}$ agar), Oat Agar (OA-25 $g.L^{-1}$ oatmeal flour, 18 $g.L^{-1}$ agar), Niger Seed Agar (NSA-100 $g.L^{-1}$ niger seed, 1 $g.L^{-1}$ dextrose, 18 $g.L^{-1}$ agar), Sabouraud Maltose Agar Yeast Extract (SMAY-10 $g.L^{-1}$ peptone, 40 $g.L^{-1}$ maltose, 2 $g.L^{-1}$ yeast extract, 15 $g.L^{-1}$ agar) and SDA. After the preparation, the media were autoclaved at 121°C for 20 min. Discs with 7 mm diameter from the inoculum were deposited in the center of Petri dishes containing approximately 20 ml of medium. Plates were incubated for 15 days in B.O.D. chamber at 28 ± 0.5°C and 12h photoperiod. The diameter of the cultures was measured in two perpendicular directions, previously marked at the bottom of each plate, every 3 days with the use of a digital caliper. Spore quantification was performed after 15 days.

Table-1 *Description of the isolates used in the present study*

Strain		Identification	GenBank ID	Source	Biological control of	Reference
Entomopathogenic fungi	B2	*Beauveria bassiana*	KU751847	Coleopteran insect	*Duponchelia fovealis*	[16]
	Bea110	*Beauveria caledonica*	KY471655	Banana tree soil	*Duponchelia fovealis*	[17]
	Isa340	*Isaria javanica*	KY488507	Banana tree soil	*Duponchelia fovealis*	[17]
	110D	*Metarhizium anisopliae*	KY471660	Banana tree soil	*Duponchelia fovealis*	[17]
Endophytic fungi	50	*Colletotrichum gloeosporioides*	MF076620	*Aristolochia triangularis*	*Botrytis cinerea*	[18]
	199	*Botryosphaeria dothidea*	MF076616	*Aristolochia triangularis*	*Rhizopus stolonifer, Botrytis cinerea*	[18]
	57	*Nectria pseudotrichia*	MF076592	*Aristolochia triangularis*	*Rhizopus stolonifer, Botrytis cinerea*	[18]
	105	*Nectria pseudotrichia*	MF076599	*Aristolochia triangularis*	*Rhizopu sstolonifer, Botrytis cinerea*	[18]

Table-2 *Effects of culture medium on the radial growth rate of the endophytic and entomopathogenic fungi. Means with equal letters within the same column present statistical equality, according to Tukey test (p <0.05)*

Culture Media	Radial mycelial growth rate (mm day^{-1})							
	Endophyte				Entomopathogenic			
	50	199	57	105	Bea110	Isa340	110D	B2
RA	3.45±0.69 ^{c}	21.62±0.00	21.62±0.00	10.77±4.07 ^{b}	2.17±1.25 ^{c}	1.67±0.40 ^{c}	3.11±0.41 ^{a}	1.61±0.16 ^{a}
OA	3.78±0.60 ^{b}	21.62±0.00	21.62±0.00	10.93±3.72 ^{ab}	4.24±0.19 ^{bc}	3.40±0.28 ^{bc}	3.39±0.17 ^{a}	2.15±0.15 ^{a}
NSA	4,73±1.01 ^{a}	21.62±0.00	21.62±0.00	11.25±2.93 ^{a}	7.01±1.23 ^{a}	6.78±1.81 ^{ab}	2.54±0.27 ^{a}	2.33±0.14 ^{a}
SMAY	4.58±1.18 ^{a}	21.62±0.00	21.62±0.00	11.05±3.43 ^{ab}	4.99±1.02 ^{ab}	9,15±0.76 ^{a}	4.05±0.48 ^{a}	2.30±0.28 ^{a}
SDA	3.26±0.76 ^{c}	21.62±0.00	21.62±0.00	10.76±4.10 ^{b}	5.68±0.83 ^{ab}	7,27±0.94 ^{a}	3.40±0.22 ^{a}	2.32±0.15 ^{a}
F-ratio	114.03	-	-	4.66	15.63	13.38	1.55	1.41
p-value	<0.0001		-	0.022	<0.0001	0.001	0.261	0.3
df	4.14	-	-	4.14	4.14	4.14	4.14	4.14

Table-3 *Effects of culture medium on the sporulation of the endophytic and entomopathogenic fungi. Means with equal letters within the same column present statistical equality, according to Tukey test (p <0.05)*

Culture	Endophyte (spores x 10^{5} mL^{-1})				Entomopathogenic (spores x 10^{6} mL^{-1})			
Media	50	199	57	105	Bea110	Isa340	110D	B2
RA	6.17±0.88 ^{b}	12.50±3.33 ^{abc}	6.17±1.09 ^{b}	4.83±1.09 ^{b}	2.95±1.11 ^{a}	2.17±0.95 ^{b}	1.18±0.20 ^{b}	0.52±0.07 ^{a}
OA	18.50±6.66 ^{ab}	28.00±7.76 ^{ab}	19.30±2.77 ^{a}	6.50±1.04 ^{b}	8.53±2.39 ^{a}	8.72±1.26 ^{a}	1.05±0.18 ^{b}	3.95±0.93 ^{a}
NSA	114.00±50.52 ^{a}	28.50±5.00 ^{a}	19.50±3.12 ^{a}	34.00±5.25 ^{a}	22.00±7.88 ^{a}	13.10±2.94 ^{a}	2.88±0.48 ^{b}	6.90±3.05 ^{a}
SMAY	37.00±8.30 ^{ab}	7.20±1.95 ^{bc}	12.80±0.88 ^{ab}	32.20±4.57 ^{a}	16.40±5.10 ^{a}	4.50±0,55 ^{b}	5.48±0.52 ^{a}	4.55±0.56 ^{a}
SDA	6.50±0.88 ^{b}	3.80±1.62 ^{c}	6.17±0.833 ^{b}	12.00±2.52 ^{b}	7.94±2.37 ^{a}	3.93±0.14 ^{b}	1.15±0.57 ^{b}	2.32±0,57 ^{a}
F-ratio	3.98	6.55	10.91	17.49	2.83	8.46	20.14	2.67
p-value	0.035	0.007	0.001	<0.0001	0.083	0.003	<0.0001	0.095
df	4.14	4.14	4.14	4.14	4.14	4.14	4.14	4.14

Table-4 *Cost of reagents for the formulation of culture medium used for growth and production of spores estimated from values found in the market in 2018*

Culture Media	Formulation cost (US$.L^{-1})					Total cost (US$.L^{-1})
	Agar	Niger	Peptone	Maltose	Yeast extract	
Niger	13,06667	0,31111	-	-	-	13,37778
Smay	10,88889	-	4,79012	20,24691	0,43259	36,35852
Cost per gram (US$)	0,72593	0,00311	0,47901	0,50617	0,21630	

A 7 mm disc was removed tangentially to the inoculum with a sterilized puncher and resuspended in 1 ml of 0.85% w/v NaCl solution with 0.01% Tween 80. The tube was shaken for 30 seconds to obtain a homogeneous suspension and the spores were counted in a hemocytometer [19]. In all experiments the germination rate was higher than 90%.

Cost evaluation

The cost of the media was calculated based on the reagents marketed by Sigma [20] and the average price provided by the Institute of Agricultural Economics [21].

Centesimal composition of the Niger seed

The protein content was determined by the Kjeldahl method, considering the nitrogen conversion factor in proteins of 6.25. The percentage of lipids was determined by the gravimetric method, using ethyl ether in a Soxhlet extractor. The moisture content was quantified by the gravimetric method of oven drying with air circulation at 105ºC until constant weight. Ash determination of the samples was performed by muffle incineration at 550°C. The crude fiber quantification was done by the Weende gravimetric method and the carbohydrate content was determined by the difference of 100% [22].

Effect of temperature and photophase on mycelial growth and sporulation

Using the NSA, temperatures of 24ºC, 28ºC and 32ºC and the luminosity regimes of 24h dark, 24h light and 12h light/12h dark were evaluated. Discs of 7 mm diameter were removed from the inoculum plate and transferred to the center of a Petri dish. The plates were incubated for 15 days in B.O.D. chamber under the different conditions tested. Growth and sporulation evaluations were performed as described above.

Statistical analysis

In order to evaluate the effect of the substrate on mycelial growth and sporulation, a completely randomized design was performed in triplicates. Mycelial growth rate in millimeters per day was performed with a simple linear regression [23]. The data were submitted to Analysis of Variance (ANOVA) at the 5% probability level and, when significance was found for the F test, a Tukey test was performed. For the evaluation of the temperature effect and photophase, a complete 3x3 factorial design was performed, with three replicates. The analyses were performed with Minitab software 18 [24].

Results and discussion

Effects of different culture medium on mycelial growth rate and sporulation

Endophytes 199 and 57 presented the highest radial growth rates in all media, 21.62 mm.d^{-1} [Table-2]. There was no statistical difference for isolates 110D and B2. Endophytic 50 presented statistically similar results in NSA (4.73 mm.d^{-1}) and SMAY (4.58 mm.d^{-1}). The SMAY medium was the best for the growth of the entomopathogens Bea110 (7.01 mm.d^{-1}) and Isa340 (9.15 mm.d^{-1}), and for the endophyte 105 (11.25 mm.d^{-1}).

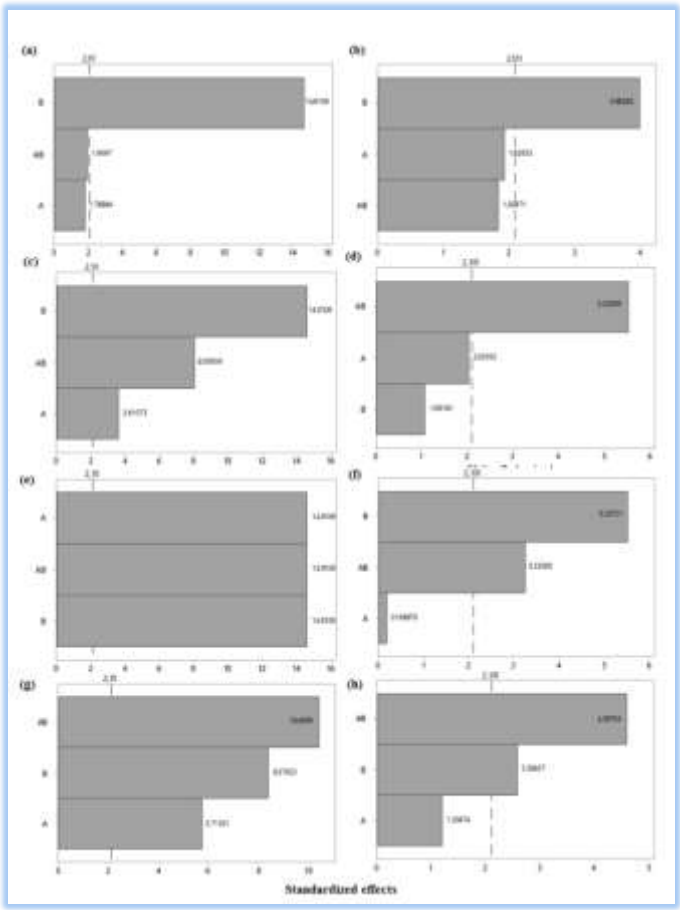

Fig-1 Pareto diagram for analysis of vegetative growth and sporulation of entomopathogenic fungi with variation in photophase conditions (24h dark, 12h light / 12h dark, 24h light) and temperature (24ºC, 28ºC and 32ºC) after 15 days of culture in NSA. Vegetative growth in the left column: (a) Bea110 (c) B2 (e) Isa340 (f) 110D. Sporulation in the right column: (b) Bea110 (d) B2 (f) Isa340 (h) 110D. Factor A: photophase; factor B: temperature. Factors above the dotted line are significant.

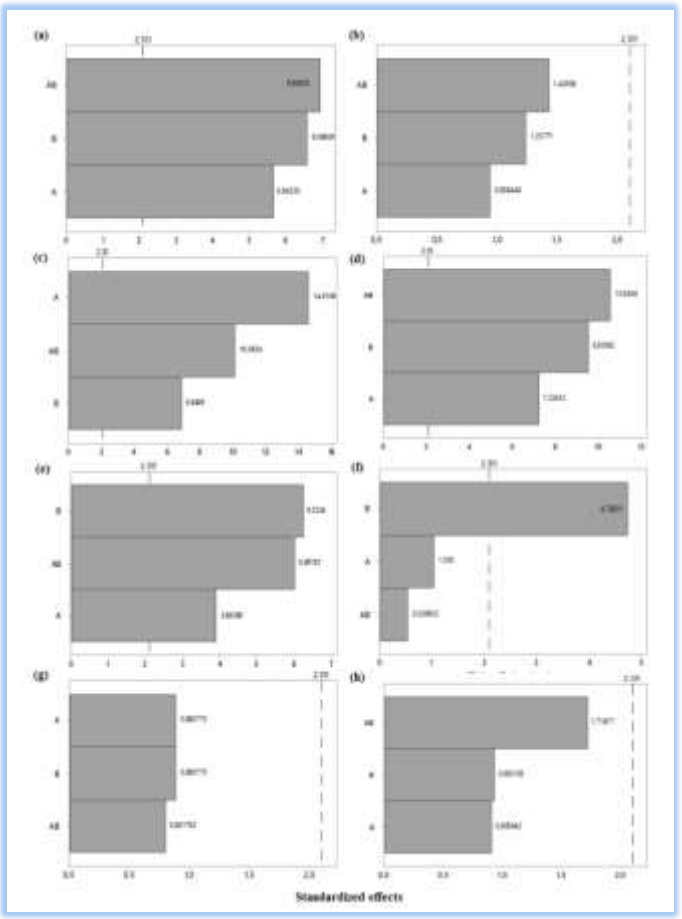

Fig-2 Pareto diagram for analysis of vegetative growth and sporulation of endophytic fungi with variation in photophase conditions (24h dark, 12h light / 12h dark, 24h light) and temperature (24ºC, 28ºC and 32ºC) after 15 days of culture in NSA. Vegetative growth in the left column: (a) 57 (c) 50 (e) 105 (g) 199. Sporulation in the right column: (a) 57 (d) 50 (f) 105 (h) 199. Factor A: photophase; factor B: temperature. Factors above the dotted line are significant.

The radial growth rate of the entomopathogens was similar when substrates were varied under the same temperature and photophase conditions. For endophytic fungi, the variation was lower, which may be associated with enzymatic machinery to hydrolyze the available plant resources, such as starch [25]. The medium containing Rice and Oats have starch as the main source of carbon, being similar to the plant from which they were isolated. On the other hand, it is known that entomopathogens produces chitinases during the invasion of its host, which may be an indication that these organisms present different enzymatic abilities to the endophytic fungi [26]. It is important that the fungi have the ability to degrade several substrates. For all the studied isolates, vegetative growth was equivalent in different nutritional conditions, which allows flexibility in the optimization of the production, as to make them adequate to future studies for field applications [27]. Another important consideration is that the fungi studied were isolated from plants, soil and insects, so the artificial culture medium tends to have more abundant available resources than in the natural habitat, which facilitates the development of the mycelium in different conditions [28]. Regarding spore production, in NSA the sporulation was significantly higher for all isolates, except for Beauveria sp. (Bea110 and B2) and *Metarhizium anisopliae* (110D) [Table-3]. Isolate 110D showed significant difference in SMAY medium, with 5.48 x 10^5 spores.mL^{-1}. The fungal sporulation process is associated with a defense response to resource depletion, which justifies the difference in spore production, even though there was no significant difference in growth. In addition to being a response to inhospitable environmental conditions, sporulation is also activated under conditions which available carbon sources are more difficult to access, such as lipids and complex polysaccharides. This allows a simultaneous sporulation to accelerate vegetative growth, justifying the good results found in NSA [29].

Cost evaluation

One of the difficulties for the production of commercially viable bio-insecticides lies in the high cost of the culture media and the production process itself due to the delay in sporulation and the risk of contamination during the process [14]. As NSA and SMAY media presented significant sporulation results compared to the others, the cost for the formulation of the medium was evaluated [Table-4]. NSA had the lowest cost, US$ 13.43 per liter, 2.7 times lower than SMAY, at a cost of US$ 36.36 per liter. Thus, Niger Seed Agar was selected for photophase and temperature evaluation.

Centesimal composition of Niger seed

Niger seed was mostly composed of lipids, 42.73%, followed by proteins (21.70%), carbohydrates (11.28%), crude fiber (10.97%), moisture (7.61%) and ash (5.41%). Similar centesimal composition was previously reported [30, 31]. In general, fungi produce different enzymes capable of lysing different carbon sources, and the fungi used in this study produced lipases capable of hydrolyzing the lipids to glycerol and fatty acids later assimilated by the cells. Lipids are even more oxidized than sugars and therefore capable of delivering more energy to the cell since they have the enzymes and the transport system needed to incorporate them [28].

Effects of different conditions of photophase and temperature on mycelial growth rate and sporulation

The temperature significantly influenced the vegetative growth and sporulation of the entomopathogenic fungi, with the exception of B2, which had a significant influence on the sporulation only of the temperature and photophase interaction [Fig-1]. The photophase and the photophase-temperature interaction significantly interfered with the vegetative growth of the isolates Isa340, 110D and B2. In sporulation, however, besides temperature, only the interaction of the factors interfered significantly for the isolates Isa340, B2 and 110D.

For the endophytic isolates 57, 50 and 105, all factors evaluated had significant effects on vegetative growth, which did not occur for isolate 199 [Fig-2]. As for sporulation, there was a significant influence of all factors for isolate 50, and only temperature for isolate 105.

In addition to requiring nutritional flexibility to be successful in the field, potential bio-insecticides and biofungicides must withstand exposure to certain extreme physical conditions such as long exposure to the Sun and large thermal amplitudes. Despite having their sporulation and vegetative growth influenced as a function of temperature and photophase variations, none of the microorganisms were completely inhibited under the conditions studied. This demonstrates resilience under different conditions, and could represent a possible ability to resist in the field up to a point of satisfactorily combating the host [32].

Conclusion

This is the first study to prove the efficiency of the NSA medium for sporulation of filamentous fungi in comparison to the media usually used. NSA is a medium commonly applied in the differential diagnosis of pathogenic yeasts [33] and had not been associated with the efficient cultivation of filamentous fungi. The optimization of nutritional and physical conditions for the cultivation of fungi with potential in pest control is a fundamental tool in the development of processes aiming commercial production. In addition, assays on different substrates and physical conditions provide subsidies for the response of microorganisms under field conditions, in order to ensure their survival and consequent success in host infection. Therefore, the results obtained in this study demonstrated that the Niger Seed Agar medium promotes sporulation superior to the means conventionally used in scientific research, as well as the economic feasibility to use it.

Application of research: This research provides an alternative substrate for large scale spore production of biological control agents.

Research Category: Industrial microbiology, environmental microbiology

Abbreviations:

BCA: Biocontrol agent, NSA: Niger Seed Agar
OA: Oat Agar, RA: Rice Agar
SDA: Sabouraud Dextrose Agar, SMAY: Sabouraud Maltose Agar Yeast Extract

Acknowledgement / Funding: This study was financed by National Council for Scientific and Technological Development (CNPq) and National Council for the Improvement of Higher Education (CAPES). Authors are thankful to Federal University of Paraná, Curitiba, Brazil

***Principle Investigator or Chairperson of research: Carolina Gracia Poitevin**
University: Federal University of Paraná, Curitiba, Brazil
Research project name or number: UFPR Banpesq 2017024695

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Department of Basic Pathology, Sector of Biological Sciences, Federal University of Paraná

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

References

[1] Crowder D. and Jabbour R. (2014) *Biol. Control*, 75, 8-17.
[2] Van Lenteren J.C., Bolckmans K., Köhl J., Ravensberg W.J. and Urbaneja A. (2017) *Biol. Control*, 63 (1), 39-59.
[3] Venugopalan A. and Srivastava S. (2015) *Biotechnol. Adv*., 33 (6), 873-887.
[4] Hardoim P., Berg G., Compant S. and Campisano, A. (2015) *Microbiol. Mol. Biol. Rev*., 79 (3), 294-306.
[5] Michaud J.P., Pell J.K. and Vega F.E. (2018) *Biol. Control*, 116, 1-2.
[6] Gunatilaka A.A.L. (2006) *J. Nat. Prod*, 69(3), 509-526.
[7] Zhao J., Wang X., Sun W., Mou Y., Peng Y. and Zhou L. (2013) *Electron. J. Biotechnol*., 16 (6), 16.
[8] El-Hawary S.S., Sayed A. M., Rateb M. E., Bakeer W., AbouZid S. F. and Mohammed R. (2017) *Nat. Prod. Res*., 31(21), 2568-2573.
[9] Faria M.R., Magalhães B.P. (2001) *Biotecnologia, Ciência e Desenvolvimento*, 22, 18-21.
[10] Vidal S. and Jaber S. (2015) *Current Science*, 109 (1), 46-54.
[11] Shah P.A. and Pell J.K. (2003) *Appl. Microbiol. Biotechnol*., 61 (6), 413-423.
[12] Van Lenteren J.C (2012) *BioControl*, 57,1-20.
[13] Banu J.G. and Rajalakshmi S. (2014) *Indian J. Plant Prot*., 42 (1),91-93.
[14] Mascarin G.M., Jackson M.A., Kobori N.N., Behle R.W. and Delalibera Jr I. (2015) *J. Invertebr. Pathol*., 127, 11-20
[15] Robl D., Sung L.B., Novakovich J.H., Marangoni P.R.D., Zawadneak M.A.C., Dalzoto P.R., Gabardo J. and Pimentel I.C.(2009) *Braz. J. Microbiol*., 40, 296-300.
[16] Poitevin C.G., Porsani M.V., Poltronieri A.S., Zawadneak M.A.C. and Pimentel I.C. (2018) *Appl. Entomol. Zool*., 53 (3), 323-331.
[17] Amatuzzi R.F., Poitevin C.G., Poltronieri A.S., Zawadneak M.A.C. and Pimentel I.C. (2018) *Insects*, 9, 70.
[18] Stuart A.K.C., Stuart R.M. and Pimentel I.C. (2018) *Int. J. Microbiol. Res*., 10 (8), 1325-1337.
[19] Kamp A.M. andBidochka M.J. (2002) *Lett. Appl. Microbiol*., 35 (1), 74-77.
[20] Sigma Aldrich (2018).https://www.sigmaaldrich.com/catalog.
[21] Instituto De Economia Agrícola (IEA) (2018) http://ciagri.iea.sp.gov.br/nia1/precos_medios.aspx?cod_sis=4.
[22] Intl, A. O. A. C. Official methods of analysis of AOAC International (1995) *Arlington, Va.: AOAC Intl. pv* (loose-leaf).
[23] Fargues J., Maniania N. K., Delmas J. C. and Smits, N. (1992) *Agronomie*, 12 (7) 557-564.
[24] Minitab Inc. Minitab 18 Statistical Software. State College, PA, 2018.
[25] Khan L., Shahzad R., Al-Harrasi A. and Lee I. (2017) *Endophytic Microbes: A Resource for Producing Extracellular Enzymes. In: Maheshwari D., Annapurna K. (eds) Endophytes: Crop Productivity and Protection. Sustainable Development and Biodiversity*,16, 95-110.
[26] Senthilraja G., Anand T., Mohankumar S., Raguchander T. and Samiyappan R. (2017) *J. Basic Microbiol*., 58(3), 1–6.
[27] DevidasP.C., Pandit B.H. andVitthalrao P.S. (2014) *Sci. World J.* (ID 273030).
[28] Carlile M., Watkinson S. and Gooday G. The Fungi. 2º. ed.: Elsevier Academic Press, 2001. p. 145-168.
[29] Keyhani N. (2017) *Fungal Biol*., 122 (6), 420-429.

[30] Singh H.J., Paroha S. andMishra R. (2017) *Int. J. Curr. Microbiol. App. Sci*, 6 (10), 2215-2226.
[31] Rao P. (1994) *Food Chemistry*, 50 (4), 379-382.
[32] Mwamburi L.A, Laing M.D. and Miller R.M. (2014) *Braz. J. Microbiol*., 46, 67-67.
[33] Lees E. and Barton R.C. (2003) *Diagn. Microbiol. Infect. Dis*., 46 (1), 13-17.