ejpmr, 2016,3(11), 334-339

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF BAVESTIN PESTICIDE ON THE GROWTH OF VESICULAR AND ARBUSCULAR MYCORRHIZAL FUNGI WITH REFERENCE TO THE DNA AND PROTEIN CONTENT

Epur Manoj Kumar Reddy*, Rajender Vadluri, Murali Krishna Thupurani and B.S. Anuradha

[1]Department of Microbiology, Chaitanya Degree and Postgraduate College (Autonomous), Kishanpura, Hanamkonda, Warangal, Telangana-India.

***Corresponding Author: Epur Manoj Kumar Reddy**
Department of Microbiology, Chaitanya Degree and Postgraduate College (Autonomous), Kishanpura, Hanamkonda, Warangal, Telangana-India.

Article Received on 28/08/2016 **Article Revised on 19/09/2016** **Article Accepted on 09/10/2016**

ABSTRACT

This paper describes the effects of the fungicide Bavastin, on mycorrihizal development in various crop plants. An attempt was made to establish the inhibitory effect of the fungicide on the net DNA and Protein content of VAM isolated from the rhizosphere soil of plants which were treated with the fungicide. Physio chemical nature of the soil was studied to evaluate of nutrient percentage of different soil samples collected. The techniques included isolation, Identification of VAM and extraction and finding DNA ratio 260/280. The highest DNA yield 46ng was found with MNJII soil sample collected from farm lands of pochampalley, Warangal District. In our investigation we identified three different genus from the soil samples collected they are Glomus, Gigaspora and Acaulospora. The highest protein content 85.3 was observed with samples collected from village pochampalley. The present study concludes that Bavestin exhibited the hazardous effects on VAM growth and directly influence the concentration of DNA and Protein.

KEYWORD: Bavastin, Glomus, Gigaspora and Acaulospora.

1.0 INTRODUCTION

Pesticides interact and react with living cells. Even though, a wide range of precautions had taken, these chemicals adversely exhibit symbiotic relationships occur between host plant and microorganisms. Vesicular arbuscular mycorrhiza (VAM) as a microbial model system is emerging as one of the frontier areas for scientists to focus on crop improvement and product development. In general crop plants avail from the VAM infection and association by greater absorption of phosphorus and water from soil.[1] It has been reported that the infection and colonization of VAM increase drought resistance in wheat plants.[2-3] In the current investigation we evaluated the side effects of pesticides on VA-mycorrhizal growth with reference to its DNA quantification. The AM fungi are the most widely studied types of mycorrhizae due abundant presence in most agricultural and natural ecosystems and play significant role in plant growth and productivity.

Now-a-days, farmers are extensively using fungicides in order to control and eliminate fungal phytopathogens. However, fungicides used on soils not only affect phytopathogens but also capable of showing adverse affects on autochthonous soil microorganisms actively involved in development of plant. The most widely studied of such soil bore microorganisms are Vesicular and arbuscular mycorrhizal (VAM) fungi.[4-7] The beneficial effects of VAM fungi on the plant growth and products can be hampered by the wide use of pesticides in agricultural systems.[8] Several studies reported that the length of external hyphal of VAM has been decreased by the fungicide treatments with the reduction of phosphorus (P) content in plants.[9]

Concerning to the effect of pesticides on VAM the current study was framed to evaluate the hazardous affects of a well known fungicide bavesten on VAM isolated from two different soil samples collected from farm lands surrounding Warangal city and farm lands of village pochampalley, Warangal District.

2.0 MATERIAL AND METHODS

Two sampling sites were selected for my research work

(I) Farm lands on (surrounding Warangal city).

(II) Farm lands (own farm land (Pochampalley), Nancharimadur, Kodakandla road, Warangal District. Location: 32.69°N 71.12°E Altitude: 412 m (1742 ft.) Above Sea Level Climate: Hot Semi-Arid Type Summer temperature: Minimum: 25° C, Maximum: 45° C.

2.1 COLLECTION OF ROOT SAMPLES

In Each sites of Selected Farmlands (Cultivated land) was divided into four different zones (MNJ I), (MNJ II), (MNJ III) and (MNJ IV). Roots and soil samples were collected from the rhizosphere of healthy plants growing in that area. The soil was dug out with a trowel to a depth of 0-15 cm. Samples were collected randomly from different zone in each site, pooled and homogenized. The collected samples were taken in sealed plastic bags, labelled and transported to the laboratory in an insulated container. Before processing, all the samples were sieved (< 2 mm mesh size) to remove stones, coarse roots and other litter, and fine roots were collected from each sample. Soil samples were airdried and stored at 4°C for further experiments.

Note: All the samples were collected from agricultural fields treated with Bavestin pesticide.

2.2 ISOLATION OF VESICULAR ARBUSCULAR MYCORRHIZA SPECIES FROM PLANT ROOTS

The following steps were Isolation of vesicular arbuscular mycorrhizal species collected from root samples.

- ❖ Fine root samples were collected and then washed with running tap water and fixed in FAA (Formalin Acetic acid).
- ❖ Roots were segmented into 1cm bits. Three replicates of 100 root bits each, selected at random were processed separately for determining the mycorrhizal intensity in the roots.
- ❖ Root bits were treated with 10% KOH solution for 30 min at 40 0 c temperatures. The concentration of KOH and time of incubation of roots depend upon the age and softness of the roots. Pour off the KOH solution and rinse the roots well in a beaker using at least three complete changes of tap-water or until no brown colour appears in the rinse water.
- ❖ After thorough washing, root bits were stained with trypan blue (0.01% trypan blue) for 24hrs at room temperature. Stained root pieces were mounted in lactoglycerol and examined under microscope for the mycorrhizal colonization and its spore's structures study.

2.3 ISOLATION OF VAM SPORES FROM RHIZOSPHERE SOIL MIXTURES

Spores were isolated from field-collected root-rhizosphere soil mixtures. Spores of arbuscular fungi were isolated by using the 'wet sieving and decanting method' described by Gerdemann and Nicolson, 1963.[10]

The following steps were made

In soil remove the coarse materials like straw, debris and rocks should be removed with a 2-mm sieve.

2.2.1 SOIL SAMPLE MIXED WITH TAP WATER

- ✓ 100 gm of air-dried root-rhizosphere soil mixture were placed into a glass container with 1000 ml of tap water.
- ✓ The root-soil mixture was vigorously mixed with a glass rod for 30 seconds. Figure 2: Soil suspension sieved
- ✓ A 10-second pause enabled to settle heavier particles and organic material, the remaining soil-water suspension were slowly poured through a set of two sieves. The sieves used are those with pores of diameters of 0.5mm (the top one) and 0.045 mm (lowest one). Most spores retain on the 0.045 mm sieve.
- ✓ The extracts were washed away and spores collected from the sieves in to Petri dishes.
- ✓ Using a microscope, spores and aggregates were picked by means of dropper and needle. Figure 4: Isolated spore picked with dropper under microscope
- ✓ Selected spores were separated with a needle. A drop or two of mountant (poly vinyl lacto glycerol) was spread on the centre of a clean and dry slide so as to hold cover slip. Spores were placed on the mountant and the cover slip was placed gently by avoiding air bubbles. Such prepared slides were labeled, allowed to dry in a dust free chamber for 3-5 days. The edge of the cover slip was sealed with clear nail polish to prevent the desiccation and entry of air bubbles and Spores were examined.

2.4 ANALYSIS OF SOIL SAMPLES

Soils constitute the weathered surface of the earth's crusts which is mixed with organic material and in which microorganisms live and plants grow. Soil testing is one of the most important tools to determine the status of plant nutrients in a field. The air dried and sieved Soil samples were analyzed for the concentration and presence of like pH, Organic carbon, macro and micro nutrients in the soil testing laboratory, Agriculture Research Center, Mulgu Road, Warangal. Soil characteristics of different sites are presented in table 1 and 2.

2.5 ISOLATION AND IDENTIFICATION OF MYCORRHIZAL SPORES

Isolation of mycorrhizal spores was carried out by wet screening methods and methods of sucrose gradient centrifugation.[11] The wet screening technique was conducted by weighing 100 g soil samples and the dissolved in 1000 ml of water then left for 10-15 minutes to allow the sediment to settle. The suspension then filtered by pouring it into filter with diameter of 40 µm, 50 µm, and 200 µm, respectively, repeated 3 times. Spores were filtered through a sieve of 50 µm and 200 µm then inserted into the centrifuge tube. A 2500 rpm speed was used to centrifuge the suspension for 5 minutes. Supernatants were collected on the top in the exhaust up to three-quarters of the tube. The remaining solution in the tube was mixed with 60% sucrose solution then centrifuged at 1200 rpm for 2 minutes. The suspension is poured into 10 cm diameter petri dishes. Further, the suspension between the water and the sugar was taken and placed on a 200 µm sieve, sieve sprayed

with water slowly to clean up spores from the remnants of attached sugar. Clean spores were placed on a petri dish diameter of 10 cm to be observed and counted under a dissecting microscope. Subsequently, population of each of mycorrhizal fungi types was counted for 100 g soil then separated on a petri dish based on shape, color and size. Based on these observations, type, population, form and spore color of the mycorrhizae were matched with standard spores.[12-13] Mounting procedure was conducted as follow left hand side of a glass object was dropped into a solution of PVLG and a portion of Melzer solution was dropped onto the right hand side. Similar spores were placed on each of the solution then each surface was covered with a cover slip. Spores were crushed by pressing the cover slip surface with a toothpick.[14] Mixtures of mycorrhizal spores were observed under compound microscope equipped with digital camera with enlargement of 100-400 times. The identification based on morphological characteristics of spores that is based on size, color, cell walls layer, ornaments, and hyphae form attached to the spores cell walls (bulbous suspensor, hyphae holder, or subtending hyphae).[15] Color change of spores in Melzer solution is one of the indicators to determine the type of spore.[15]

2.6 ISOLATION OF VESICULAR AND ARBUSCULAR MYCORRHIZA GENOMIC DNA

The genomic DNA of VAM was extracted from five to seven days old fungal cultures grown either in liquid broth medium. The fungal mass from the culture broth was obtained by filtering the culture broth through a 10 ml syringes containing glass wool that will allow the broth to pass through, while retaining the fungal mass. The fungal mass obtained was placed in a 2ml tube containing a ceramic pestle, 60–80 mg sterile glass beads (425–600 µM, Sigma) and lysis buffer (100 mM Tris HCl [pH8.0], 50mM EDTA, 3% SDS). Homogenization of fungal mass was done twice in a FastPrep®-24 tissue homogenizer (MP Biomedicals, USA) at 6 M/S for 60 sec. The resulting fungal tissue homogenate was centrifuge at 13,000 rpm for 10 min and supernatant was transferred to a fresh microcentrifuge tube. To the supernatant, 2 of RNase A (10mg/ml) was added and incubated at 37°C for 15 min. After the RNase A treatment, equal volume of phenol: chloroform: Isoamyl alcohol (25:24:1) was added and mixed well, followed by centrifugation at 13,000 rpm for 10 min (Note: this step can be repeated once more to completely get rid of proteins/cell debris). The upper aqueous layer was taken in a fresh micro centrifuge tube and then equal volume of 100% ethanol was added. Following precipitation at -20°C for 30 min, the whole content was centrifuged at 12,000 rpm for 10 min to pellet down the DNA. The DNA pellet was washed with 70% ethanol and centrifuged at 12,000 rpm for 5 min. The DNA pellets were air dried and dissolved in 1× TE buffer.

2.7 QUANTITY AND QUALITY DETERMINATION OF DNA

The quantity of the extracted DNA was determined by measuring the absorbance at 260 nm using Thermo Scientific Nano Drop 1000 spectrophotometer. All the DNA isolated samples were sent to Centre for Cellular and Molecular Biology (CCMB), Hyderabad to evaluate the purity of the DNA isolated from the VAM fungi by Nano Drop Technology.

2.8 PROTEIN EXTRACTION FROM VAM CULTURES

2.8.1 Organic Medium (OM)

Media used for the extraction of protein from the VAM cultures comprises of 1% glucose (10 g/L), 0.1% Peptone (1g/L), 0.01% Yeast Extract (0.1g/L), 0.1% KH2PO4 (1 g/L), 0.03% MgSO4 7H20 (0.3g/L) or MgSO4 anhyd. (0.146 g/L). All the chemicals are analytical grade and purchased from SD fine chemical Laboratories, Mumbai, India.

2.8.2 Keeping spores in Silica-Gel

Sterilize silica gel in small capped glass vials in 180^{o} C oven for 1 day, Sterilize 5% (w/v in H_2O) powdered milk (autoclave), Add approx. 4ml of milk to each petrie-plate containing sporulating fungal culture • If there is a lot of mycelium, filter the suspension through gauze. Add 0.2ml of spore suspension to each vial containing silica gel. Let air-dry for one week in vials with loosely screwed caps, Tighten caps for permanent storage. To revive culture, inoculate plates with 3-4 pieces of silica gel Protein Isolation from Frozen Ground Tissue Protein Isolation Buffer (PIB) 10 mM Tris-HCl pH 8.0, 1mM EDTA, 2% PVPP. Shake buffer before use. Add proteases inhibitors (50ul each in 10ml): chymostatin, aprotinin, leupeptin plus 500 ul PMSF in methanol. Use 5ml of PIB per 2 g frozen tissue. Pour over frozen tissue and allow it to thaw on ice. Centrifuge at 8000 rpm for 30 min at 4^{o} C. Collect supernatant and add equal volume of acetone (freeze O/N). Centrifuge for 30 min at 6000-7000 rpm (4^{o} C). Pour off supernatant and allow pellet to air dry. Resuspend pellet in 1 ml TE (10 mM Tris-HCl, 1mM EDTA, and PMSF) (crude extract).

3.0 RESULT AND DISCUSSION

3.1 Physico–chemical Properties of Soil

The soil samples collected from different areas were screened for their levels of different elements and different properties of soil. The results are represented in table 1. The pH of the sample of two different regions ranged between 8.0-8.2. Other properties such as electric conductivity.

Table 1: Physico-chemical properties of different rhizosphere soils of farm lands surrounding Warangal city and Pochammpalley area, District Warangal.

Samples			pH	EC (ds/m)	Organic (C) (%) P (mg/g)		Macro and Micro nutrients			
						Fe (mg/g)	K (mg/g)	Zn (ppm)	Cu (ppm)	Mn (ppm)
Study site	Farm lands (I)	MNJ-I	8.2	0.21	0.15	1.10	52	178	6.05	0.82
		MNJ-II	8.1	0.33	0.36	1.19	39	171	12.51	0.56
		MNJ-III	8.0	0.28	0.18	1.03	40	166	26.81	0.40
		MNJ-IV	8.0	0.41	0.22	1.08	38	186	33.56	0.72
	Farm lands (II)	MNJ-I	8.2	0.32	0.20	1.18	35	254	9.14	0.58
		MNJ-II	8.0	0.25	0.15	1.24	39	228	7.26	0.77
		MNJ-III	8.0	0.35	0.17	1.08	40	198	17.88	1.23
		MNJ-IV	8.1	0.28	0.26	1.11	45	243	15.39	0.91

3.2 ISOLATED OF VESICULAR ARBUSCULAR MYCORRHIZA FUNGI

The current study we have isolated highest number of VAM spores 105 was found in the area grouped as MNJ-II of own farm land present in pochampalley, Warangal District. Whereas, the highest number of VAM spores 96 are noticed from MNJ-III of farm land present surrounding of Warangal city. The results were represented in the table 1.

Table 2: Enumeration of Vesicular Arbuscular Mycorrhiza Fungi per 100 gm of soil samples.

Samples			Number of VAM spore population per 100 gm of soil						
			Sample-1	Sample-2	Sample-3	Sample-4	Sample-5	Total	Type of VAM
Study site	Farm lands (I)	MNJ-I	12	15	15	19	18	79	*Glomus sps, Gigaspora*
		MNJ-II	8	11	20	14	15	68	*Glomus sps, Gigaspora*
		MNJ-III	19	23	12	18	24	96	*Glomus sps,*
		MNJ-IV	15	09	19	11	09	63	*Gigaspora, Acaulospora*
	Farm lands (II)	MNJ-I	18	15	07	09	15	64	*Glomus sps,*
		MNJ-II	25	20	28	13	19	105	*Glomus sps, Gigaspora*
		MNJ-III	11	12	17	22	21	83	*Glomus sps, Gigaspora*
		MNJ-IV	16	08	12	14	09	59	*Glomus sps, Gigaspora*

3.3 ISOLATION AND IDENTIFICATION OF MICORRHIZA

Isolation and identification of the varieties of VAM from the soil samples collected from rhizosphere layer was carried out from the plants growing in Warangal city surroundings and as well from the own cultivation land present in the pochampalley, District Warangal. In the current study the mycorrhizal fungi were classified based on VAM genus. Microscopic examination was conducted to observe and determine the different genus of VAM spores from the soil. In accordance to our investigation data we identified three different genus from the soil samples collected they are i.e. Glomus, Gigaspora and Acaulospora (Table 2).

3.4 ISOLATION OF VESICULAR AND ARBUSCULAR MYCORRHIZA GENOMIC DNA

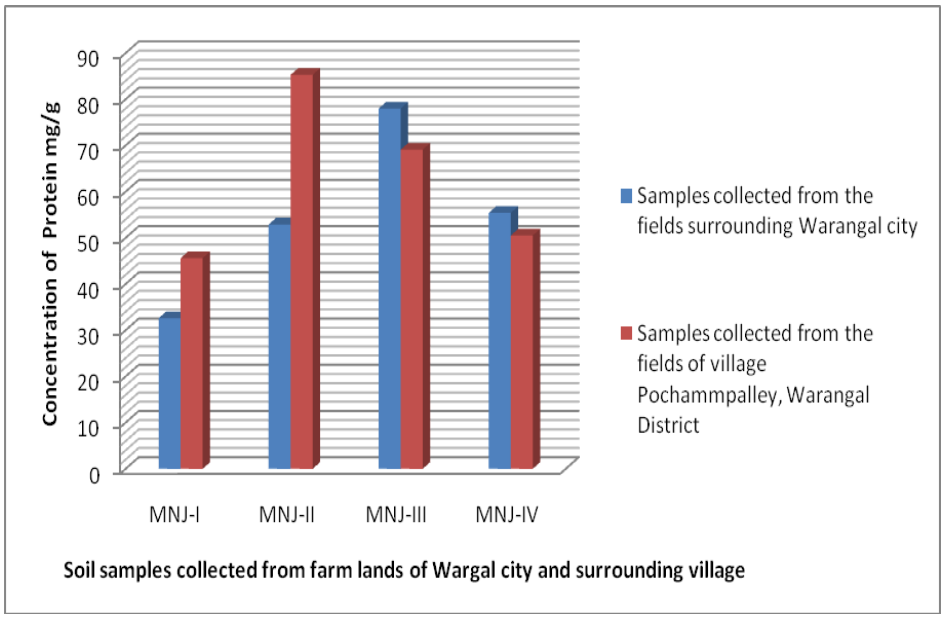
The fungal DNA extraction method yielded high quality molecular weight DNA from the fungal samples collected. The total genomic DNA yield was ranged 10 ng – 46 µg/200 mg of fungal mass (See table 3). The highest DNA yield 46ng was found with MNJII* (See table 3) soil sample collected from farm lands of pochampalley, Warangal District. The samples collected from the farm land surrounding Warangal District resulted in low yield of DNA compared with that from samples collected from farm lands of pochampalley.

Table 3: Purity of DNA 260/280 ration per 200 mg of VAM fungi samples collected from different regions of Warangal.

S. No	Sample	260/280	Ratio	DNA conc.
1	MNJ-I#	9.60	~1.2	19ng
2	MNJ-II#	5.53	0.8	07ng
3	MNJ-III#	12.97	1.7	30ng
4	MNJ-IV#	10.48	1.5	25ng
5	MNJ-I*	10.66	1.6	32ng
6	MNJ-II*	14.52	1.8	46ng
7	MNJ-III*	8.12	1.1	35ng
8	MNJ-IV*	7.81	1.0	30ng

3.5 ESTIMATION OF PROTEIN ISOLATED FROM VAM CULTURES

The study was designed to determine the protein content in the VAM samples isolated from soil samples collected from different farm lands. According to our study, the protein content was found high in MNJ-II VAM cultures related to the soil sample collected from Pochampalley village Warangal District. The protein content of VAM culture samples cultured from the soil samples collected from the fields of Warangal District showed low protein content. The results are represented graph 1.



Graph 1: Estimation of protein using Lowrry method from the VAM samples isolated from the farm lands of Warangal city and Pochampalley village, Warangal District

4.0 CONCLUSION

The present study concludes that Bavestin exhibited the hazardous effects on VAM growth and directly influence the concentration of DNA and Protein. In future we have decided to investigate the effects of some other pesticides which are common use by farmers in agriculture.

FUNDING

The current research work was funded by University of Grants Commission New Delhi. The research file number is F.NO. 6086/2014-15(MRP-SEM/UGC-SERO)

ACKNOWLEDGEMENT

We sincerely, thank to Dr. C.H.V. Purushotham Reddy, Chairmen of Chaitanya Group of Colleges, Kishanpura, Hanamkonda, Telangana, for providing laboratory to carry out the work. We are grate to University of Grants Commission (UGC), New Delhi and South eastern Regional Office (SERO), Hyderabad our accepting our research proposal and granting the funds. We also thank to Late Arakala Thirupathiah for his kind suggestions during the research work.

REFERENCES

1. Zakaria MS, Paul Blackwell, Lynette K, Abbott and Paul Storer. Direct and residual effect of biochar application on mycorrhizal root colonization, growth and nutrition of wheat. Soil Research, 2010; 48(7): 546-554.
2. AlKaraki GN. Benefit, cost and water- use efficiency of Arbuscular mycorrhizal durum wheat grown under drought stress. Mycorrhiza., 1998; 8(1): 41-45.

3. Ghazi, Al- Karaki, McMichael B, John Zak. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Ycorrhiza, 2004; 14(4): 263-269.
4. Barea JM, Azcon R, Azcon-Aguilar C. Mycorrhizosphere interactions to improve plants fitness and soil quality. Antonie van Leeuwenhock., 2002; 81(1-4): 343-351.
5. Jeffries P, Gianinazzi S, Perottos S, Turnau, K, Barea JM. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils., 2003; 37(1): 1-16.
6. Arriagada C, Maguel D, Cornejo P, Soto J, Sampedro, I, Ocampo J. Effects of the coinoculation with saprobe and mycorrhizal fungi on Vaccinum corymbosum growth and soil soil enzymatic activities. J. Soil Sci. and Plant Nutr., 2012; 12(2): 283-294.
7. Tanwar A, Aggarwal A, Kadian N, Gupta A. Arbuscular mycorrhizal inoculation and super phosphate application influence plant growth and yield of Capsicum annum. J. Soil Sci. and Plant Nutr., 2013; 13(1): 55-66.
8. Trappe JM, Molina R, Castellano M. Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides. Ann. Rev. Phytopathol., 1984; 22(1): 331-359.
9. Kling M, Jakobsen I. Direct application of carbendazim and propiconazole at field rates to the external mycelium of three arbuscular mycorrhizal fungal species: effect on 32P transport and succinate dehydrogenase activity. Mycorrhiza., 1997; 7(1): 33-37.
10. Gerdemann JW, Nicolson TH. "Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting." Trans. Brit. Mycol. Soc., 1963; 46(2): 235-244.
11. Walker C, Mize CW, Mcnabb JrHS. Population of endogonaceus at two location in Central Iowa. Canadian J. Of Bot., 1982; 60(1): 2518-2529.
12. Schenck NC, and Perez Y. Manual for The Identification of VA Mycorrhiza Fungi 3rd Edition. Gain sville: Synergistic publication., 1990.
13. Brundrett MC, Bougher N, Dell B, Grove T, Malajczuk N. Working with Mycorrhizas in Forestry and Agriculture. Canberra: Australian Centre for International Agricultural Research., 1996; 374.
14. Nusantara AD, Bertham YH, MansurI. Bekerja dengan Fungi Mikoriza Arbuskula. First Published by SEAMEO BIOTROP Southeast Asian Regional Centre for Tropical Biology., 2012.
15. INVAM. International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi. West Virginia University, Morgantown, West Virginia. URL: http://invam.wvu.edu/the-fungi/species-descriptions (diacces 30 Juli 2015)., 2015.