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**A new species of Basidiomycetes for the fungal diversity of Morocco:  
*Inocybe squarrosa* and *I. rufuloides***

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**ABSTRACT**

*Two species of the Basidiomycetes Macromycetes were studied and described: Inocybe squarrosa Rea. 1915 and Inocybe rufuloides Bon 1984 in this paper. Surveys were done in the Jerada mine site, allowed to note the details of the encountered fungal species. In the laboratory, the macroscopic and microscopic identification criteria of these species were determined. The systematic position and ecological importance of these species have been discussed in this work. The obtained results were part of the contribution to the determination of the fungal diversity in Morocco, which remains incomplete till now.*

**Keywords:** Morocco, Jerada, Fungi, Basidiomycota, Inocybaceae.

**INTRODUCTION**

Mycological studies of mushrooms in the Northeastern region of Morocco and particularly at the Jerada mine site are still rare. So far mycological work in this region were about only certain groups such as Tricholomataceae, Bolbitiaceae, Pezizales and Coprinoides<sup>8,9,10,11,12</sup> although the site has a high fungal resource potential.

The genus *Inocybe* was placed mostly in the Cortinariaceae family<sup>37</sup> its transfer to the Inocybaceae family was justified by the molecular phylogenetic studies that showed she is the sister of Crepidotaceae family<sup>29,30</sup>. These authors also suggested that the fact that representatives of Inocybaceae are ectomycorrhizal, allows the separation significantly from those of the Crepidotaceae. The *Inocybe* genus including the *Inocibium* (Earle) Sing subgenus is divided into 11 sections according to Singer<sup>38</sup>. However, Martin<sup>31</sup> did not recognize only eight sections are separated primarily based on the form of spores, the presence and extent of cystids on the stipe.

According to the available mycological studies, the *Inocybe* genus has a large number of species and the determination of these species is always difficult and often uncertain since the distinguishing features are rare. The mycologist Kuhner considers it as infernal. It is certain that only an examination of both the microscopic and macroscopic characters allow a reliable determination, although doubts often remain<sup>26</sup>. Some authors found little usefulness in perspective of a microscopic character, since very few species are determinable by means of these characters. Kuhner and Romagnesi<sup>22</sup> recommended not neglecting the macroscopic characters because of some species belonging to the as the *Inocybe* genus do not present a great wealth of microscopic characters. While, Bon<sup>5,6</sup> have used the microscopic characters to divide the genus into several groups and the *Inocybe* genus was kept in the Cortinariaceae family. Currently, according to the new systematic classification based on molecular phylogeny, this genus was transferred to the new Inocybaceae Family<sup>31</sup>. It's represented by more than 500 species in the world<sup>20</sup> and 62 its species were reported in Morocco<sup>32,33,35,36</sup>.

This work aims to continue the studies on fungal flora of the Beni Yaala forest, the mining site of Jerada, we started since 2008, studying two species of the *Inocybe* genus: *I. squarrosa* and *I. rufuloides*.

## MATERIALS AND METHODS

Jerada, located in Northeastern Morocco, includes the forest of Beni Yaala which is an artificial plantation of Aleppo pine (*Pinus halepensis*) on red Mediterranean soil that is leached, crusty and brown<sup>1</sup>. Jerada city is anthracite mine located in the foothills of the chain Horsts. The climate is arid to semi-arid and the annual rainfall is 518 mm<sup>2</sup>. Besides its socio-economic role, the forest of Beni Yaala plays an ecological role. Concerning the mycoflora mushrooms grow during different periods when climatic conditions become favorable on craft waste, craft galleries and coal ramps transformed into isohumic soils<sup>8</sup>. Surveys carried out from January to February 2013 in the Jerada mine site (forest of Beni Yaala of *Pinus halepensis*) have allowed the harvest of two species belonging to the the *Inocybe* genus: *I. squarrosa* and *I. rufuloides* for the first time in this region. In this site, the basidiocarps were photographed and some macroscopic details were noted. Then, the basidiocarps were taken to the laboratory to complete the macroscopic descriptions, shape, color and other features of the cap and the stipe. This study was complemented by a microscopic description of spores and cuts at the hymenium, the cuticle, flesh and stipe. The size of the cystidia and basidiospores was also measured. Microscopic observations were effected by the tap water. The determination of the studied species was achieved by the determination keys<sup>22,6,7,26,37</sup> and also to the data published by Maire and Werner; Malençon & Bertault ; Kuyper; Gerhault ; Gerault ; Iotti *et al.* ; Ouabbou *et al.*<sup>32, 33,24,25,16,15,18,35,36</sup>.

## RESULTS

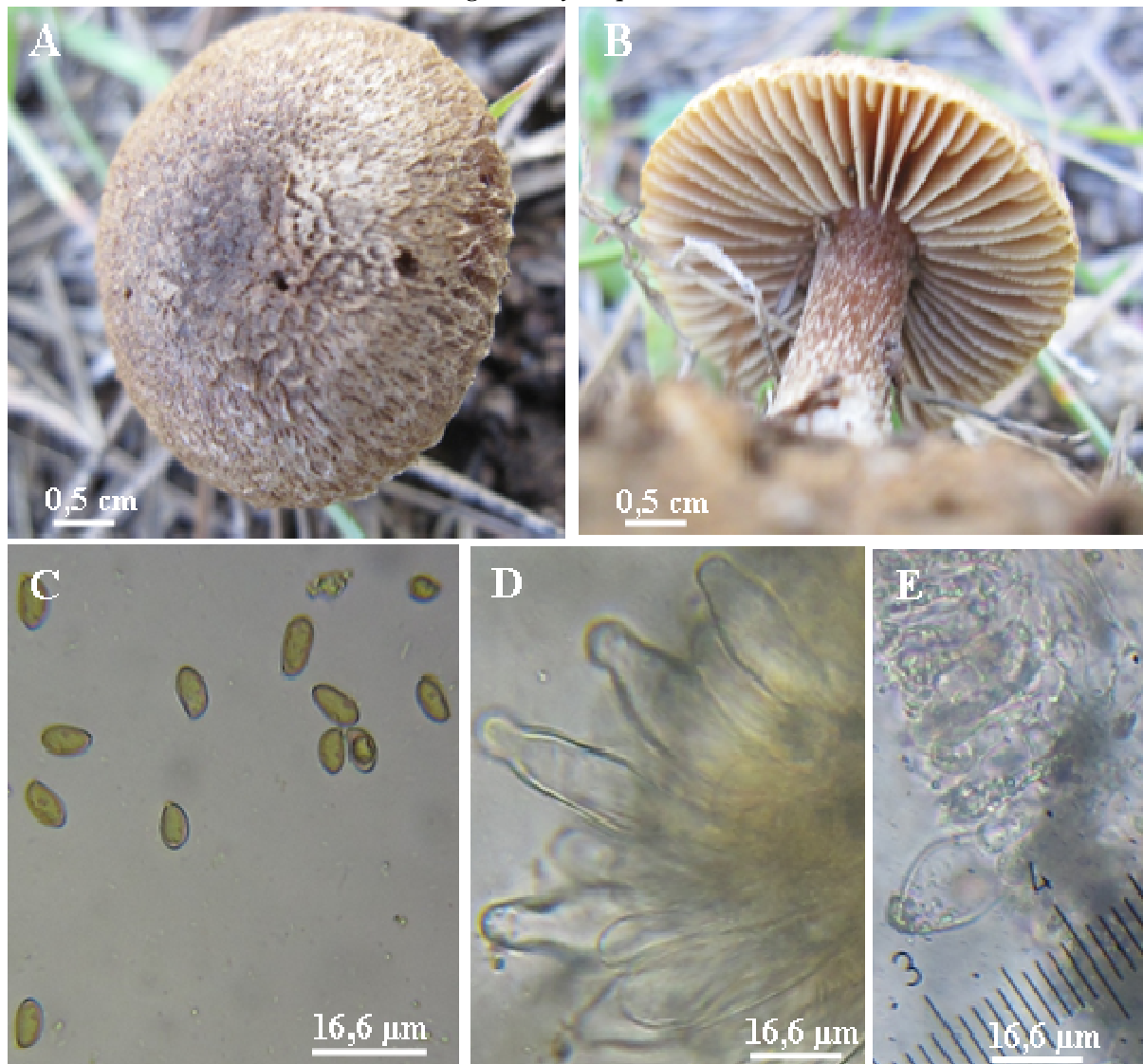
Two species have been described in this study:

- *Inocybe squarrosa* Rea, 1915, Synonym: *Inocybe squarrosa* var. *macrosperma* Esteve-Rav. & Tabarés, Revta Catal. Micol.22: 145 (1999).

This species was encountered in January 27<sup>th</sup>, 2013, on sandy soil with needles and on small dead branches of *Pinus halepensis* in the forest of Beni Yaala of the Jerada mine site (Fig.1A).

**Cap 1.5 to 3 cm in diameter, hemispherical, dark brown cuticle under a thin white veil**, fibrillose initially and then quickly separated into small flakes, mostly raised, almost circular scales at the center, greater or lesser appressed outward, becoming brownish to grayish brown background, brown to blackish ochracé at the center (Fig. 1B). Stipe: 2-4 x 0.5-0.8 cm, fibrillose or subsquamulose barely covered with dense bloom in low and high, slender, cylindrical, equal or slightly swollen at the base, full and bulbous (Fig. 1B). Flesh: beige and pale ochraceous. Odor very low sperm. Print spores, brown ocher to brown.

Basidia, 26-40 x 8-10 µm, close to clavate, 4-spored sometimes 2-spored, curled at the base. Basidiospores, 10.5 x 6 µm, varying shapes, ovoid to subamygdaliformes, smooth, slightly thick-walled (Fig. 1C). Cheilocystidia: abundant dispersed, similar to pleurocystidia, but often larger, thinner walled, up to 2 µm wide, sometimes overcome amorphous globules (Fig.1D). Pleurocystidia: 50-70 x 14.5 to 28.5 µm, scanty, dispersed polymorphic subfusiform, sublageniform, bulging closely stalked and short neck, sometimes clavate, curled at the base, thick-walled towards apex, up to 2-3.5 µm wide, pale yellow to bright, sometimes subhyaline with some fine crystals and sometimes viscous exudate at the apex (Fig. 1E). Caulocystidia: unobserved. Lamellar trama: regular, consisting of hyphae rather loosely arranged, heterogeneous, swollen-bellied in, spindle-clavate to globose, often large cells. Loops: present in all the tissues. Cheiloparacystidia: 23 x 9 µm, abundant, widely clavate to subglobose, curled at the base, thin-walled.

Fig. 1: *Inocybe squarrosa*

Basidiocarp *in situ* (A); basidiocarp released from its substrate showing the shape, color and ornamentation of the stem, as well as the shape, color and the type of insertion of gills (B); basidiospores microscopic structure (C); microscopic structure of cheilocystidia and cheiloparacystidia (D); microscopic structure of pleurocystidia (E).

The microscopic observations are made in water at 400 x.

*-Inocybe rufuloides* M. Bon, 1984, synonym: *Inocybe rufuloides* var. *rufuloides* Bon, 1984.

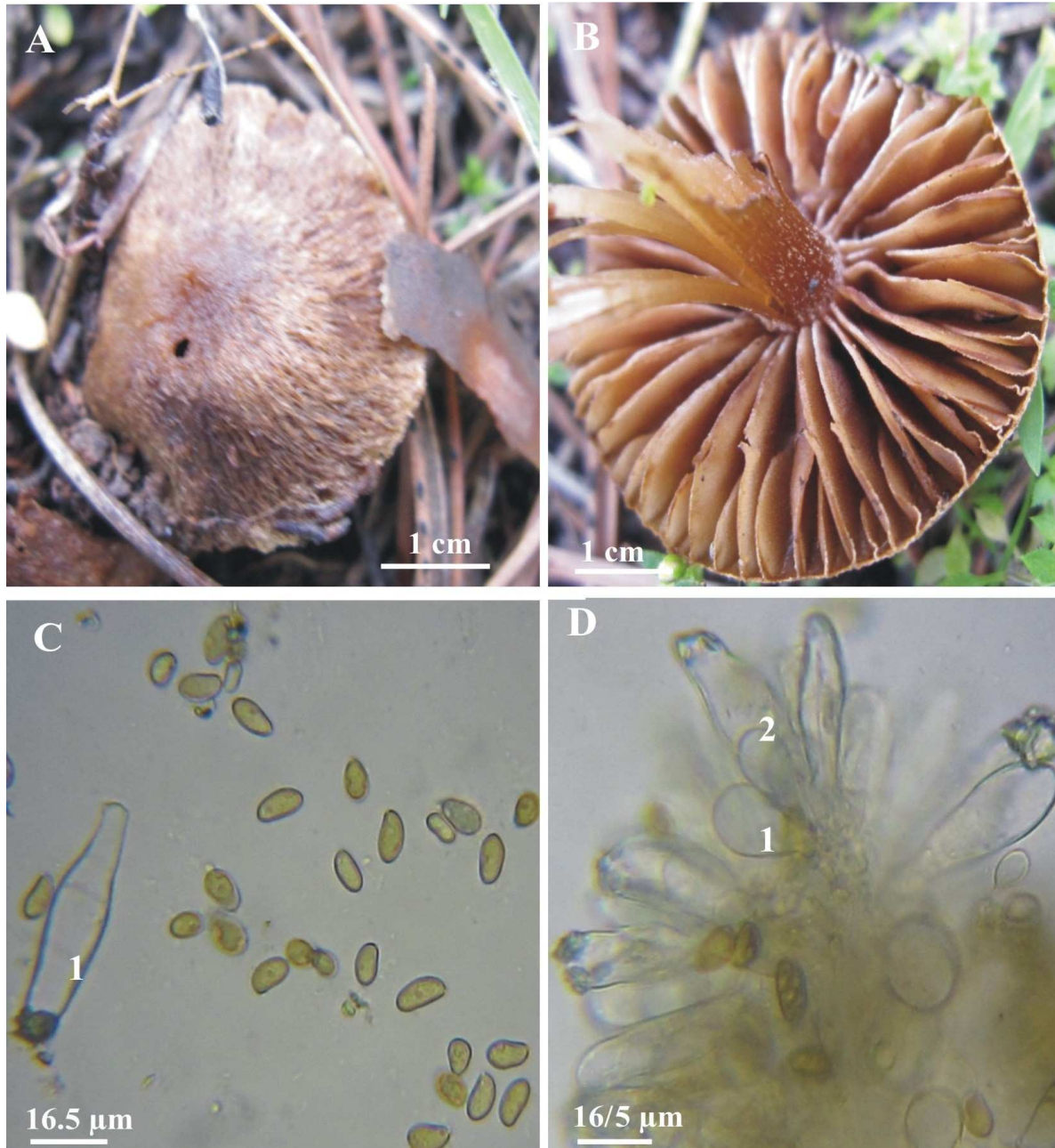
This species was encountered in February 23<sup>th</sup>, 2013, on muddy and sandy soils with needles and small pieces of wood, under *Pinus halepensis*, in the forest of Beni Yaala of the Jerada mine site (Fig. 2A).

Cap: 2-4 cm in diameter, convex, then conico-campanulate, maintaining a broad central umbo; margin plane with evanescent whitish appendiculate; surface dry radially fibrose (Fig. 2A). Gills: narrowly adnate, brownish gray and cream rusty and finally brown reddish at the end sombre, edges whitish. Stipe: 2-4 x 0.5-0.7 cm, slightly bulbous, pruinose at the top, fibrillose also subconcolore to cap, sombre ochraceous to reddish in the pruina up more pinkish reflection (Fig. 2A). Flesh: pinkish cream, darker in the stipe. Odor low sperm.

Basidia: 6-8 x 26-32  $\mu\text{m}$ , quadrisporic, hyaline, clavate, looped to the base (Fig. 2B). Spores print: brown to brown ocher. Spores: 9-13 x 5-6.5  $\mu\text{m}$ , ovoid to subamygdaliform in profile sometimes with slight adaxial depression, with slightly thick wall, yellowish brown, apiculus small but prominent (Fig. 2C). Cheilocystidia: abundant dispersed, similar to pleurocystidia, but slightly larger, thinner walled, up to 2  $\mu\text{m}$  wide, sometimes overcome amorphous globules (Fig. 2C).

Pleurocystidia: 50-70 x 14.5 (25.5  $\mu\text{m}$ ), scanty, dispersed polymorphic subfusiform or utriform, crystalliferous, lageniform closely pedicellate and short neck, sometimes clavate, curled at the base, thick-walled towards the apex, up to 2-3  $\mu\text{m}$  wide, pale yellow to bright, (Fig. 2C). Paracystidia: 23 x 9  $\mu\text{m}$ , abundant in coward clusters, subglobose to broadly clavate, curled at the base. Pileipellis in cutis, hyphae formed looped the thin-walled. Loops present in all the tissues.

**Fig. 2: *Inocybe rufuloides***



Basidiocarp *in situ* (A); basidiocarp relieved of its substrate showing the pinkish reflection top of the stem and leaves to pale whitish (B); microscopic structure of basidiospores (C) microscopic structure of pleurocystidia (C1); microscopic structure of cheilocystidia (D2) cheiloparacystidia (D1).The microscopic observations are made in water at 400 x.

## DISCUSSION

According to the microscopic characters, some authors have divided the *Inocybe* genus into 3 sub genera<sup>5</sup>. Species with smooth spores belong to two sub-genera: *Inocybe* and *Inosperma*. The first presents the cystids on the faces of the blades (pleurocystides) and the second does not. The species of the *Clypeus* genus possess gibbous spores.

The studied species have pleurocystidia (cystidia on the face of the gills) and smooth spores, so they belong to the *Inocybe* subgenus.

Macroscopic characteristics (curtain is clearly visible on the cap and stipe in adult subjects, striking ornamentation of scales of tip) and microscopic (smooth spores, cheilocystidia metuloid variable forms and presence of caulocystidia, 4-spored and 2-spored basidia) identified as *Inocybe squarrosa*, largely consistent with those noted by Esteve-Raventós *et al.*<sup>13</sup>, Lachapelle<sup>26</sup> and Gerault<sup>15</sup>, especially the appearance of its cap and his atypical cystidia and 2-spored basidia. There are small differences in color and ornamentation of the cap and the presence of flakes at the base of stipe. This polymorphism can be explained by the difference in climatic conditions and the age of the carpophores<sup>3</sup>. *Inocybe squarrosa* has never been reported in Morocco, it might be considered new species for Moroccan mycoflora.

There are two species with a spiky cap resembling to *I. squarrosa*: *I. hystrix* and *I. griseoscabrosa*. The first presents a bristling uniformity in stipe and in cap, which is typical cystids of the genus. The second, native of North America, has a very long stipe fibrillose and very long cystidia<sup>14</sup>. *I. griseoscabrosa* was also encountered in Sweden<sup>25</sup>.

*Inocybe cincinnata* is also closer to *I. squarrosa*, sometimes it is difficult to distinguish, but the former is more thin, having a cap less bristling and does not have any purplish neither on the foot nor at the flesh<sup>39,27</sup>. *I. squarrosa* was harvested under willows in muddy places, and under Poplars in France<sup>39</sup>, while in Morocco it was harvested under *Pinus halepensis*.

*Inocybe rufuloides* practically devoid of cystidia on the stipe, including this one more woolly-fedora cap, uncombed at the margin, without olive color of gills. Under the microscope, there is presence of paracystidia, which should be added to the cystidia thin-walled, presence of marginal hairs and large spores with summit mostly subobtus.

Macroscopic and microscopic characteristics previously mentioned are consistent with those cited by Bon<sup>4</sup>, Courtcuise and Duhem<sup>7</sup>, Neale Bougher *et al.*<sup>34</sup> and Tanchaud<sup>40</sup>. It was found that the marginal bristles indicated by the authors, are occasionally present and the spores are larger than those reported by Bon<sup>4</sup> in the initial diagnosis, which confirms the identification *I. rufuloides*. In Western Australia, *I. rufuloides* is an exotic species and exclusively associated with *Pinus* can be recognized by its presence in pine plantations and its morphological characteristics<sup>34</sup>.

- Pileus, bronze to dull brown color, radially fibrillose with whitish fibrils when it is young.
- Pale lamellae.
- Pinkish top of the stipe.
- Sperm odor grassy.
- Smooth spores, ellipsoid to subamygdaliforms.
- Cheilocystidia and pleurocystidia metuloid.

*I. rufuloides* is considered a species ectomycorrhizal of *Pinus* species in Europe, for example *Pinus pinea*<sup>18</sup>, Bon<sup>4</sup> reported that the species form a symbiotic association with other species, for species of the genus *Picea*. The *I. rufuloides* specimens of Western Australia have very little terminal differentiated cells on the surface of the stipe, with only cauloparacystidia and rare caulocystidia. Further evidence of the morphological variability of *I. rufuloides* is that the harvests of Western Australia are much less reddish-brown against the specimens presented by Vesper and Richter<sup>41</sup>.

*Inocybe rufuloides* as it was described by Bon<sup>4</sup>. It has been illustrated and revised in Europe by Kuyper<sup>24</sup>. This fungus is widespread in coastal sand dunes in Italy. The carpophores begin to grow with the arrival of autumnal rains and sometimes continue without interruption throughout the winter season. Development can be observed until late spring if weather conditions remain favorable (wet weather and little wind) according to Lantieri *et al.*<sup>28</sup>. In France, *I. rufuloides* is reported under conifers and tens of specimens were observed in sandy environments<sup>40</sup>. *Inocybe rufuloides* species are very common in Iberian Peninsula under *Pinus* in the sand and calcareous soils, which differs from *I. aurantiobrunnea* under microscope. The cystidia are lageniform with thicker walls (1.5-3 µm) and abundant<sup>4</sup>.

In Morocco, Maire & Werner<sup>32</sup>, Malençon & Bertault<sup>33</sup> and Ouabbou *et al.*<sup>35,36</sup> have identified 62 species of the genus *Inocybe*: *I. abjecta*, *I. appendiculata*, *I. asterospora*, *I. boltoni*, *I. bongardi*, *I. bresadolae*, *I. brunnea*, *I. calospora*, *I. cervicolor*, *I. cincinnatula*, *I. corydalina*, *I. curreyi*, *I. decipientoloides*, *I. descissa*, *I. varbrunneoatra*, *I. dolichospora*, *I. dulcamara*, *I. eutheles*, *I. fastigiata*, *I. fastigiata f. argentata*, *I. fastigiata var. cerina*, *I. fastigiata f. subcandida*, *I. flocculosa*, *I. friesii*, *I. geophylla*, *I. grammonocephala*, *I. grammopodia*, *I. griseovelata*, *I. guasapata*, *I. gymnocarpa*, *I. haemacta*, *I. halphila*, *I. hirtella*, *I. jurana*, *I. kookei*, *I. lacera*, *I. maculata*, *I. margaritispota*, *I. mixtilis*, *I. oblectabilis*, *I. oblectabilis f. decemgibbosa*, *I. obscura*, *I. ochrofulva*, *I. olida*, *I. orbata*, *I. pallida*, *I. paludinella*, *I. praetervisa*, *I. pudica*, *I. pyriodora*, *I. pyriodora f. incarnata*, *I. pyriodora var. scabra*, *I. queletii*, *I. reseipes*, *I. rhodella*, *I. rufula*, *I. sambucina*, *I. scabelliformis*, *I. tigrina*, *I. tristis*, *I. umbrina*, *I. valida*, *I. xanthomelaena*, and five undetermined species (*Inocybe* sp.). Thirty-one of these species have been encountered in the Middle Atlas, twenty-seven in Tangier, nineteen in the Rif, nineteen in Mamora, eight in Larache, three in the High Atlas, two in the Oulmes forest (East central Plateau) and one in the Casablanca region. Species studied here (*I. rufuloides* and *I. squarrosa*) that were harvested in the forest of Beni Yaala of Jerada under *Pinus halepensis*, were not included with these species and therefore are new to the fungal flora of Morocco.

The *Inocybes* are a fungi of muscarinic activity, namely they are responsible for a clear and dramatic syndrome, the muscarinic syndrome, whose main features are: bradycardia (low heart rate), hypotension, a narrowing of the pupil, generalized hypersecretion<sup>24</sup>. The toxin responsible for this syndrome has cholinergic alkaloid properties. This toxin is similar to acetylcholine, which functions as a neurotransmitter in the central and peripheral nervous system in many organisms including humans<sup>17</sup>. There are also species of the genus *Inocybe* which contain psilocybin, which is a hallucinogenic substance<sup>24</sup>.

The *Inocybes* frequently develop in parks and along roads, increasing the risk of their use by children or pets<sup>17</sup>.

### CONCLUSION

Two species of Basidiomycetes macromycetes that are encountered in the Jerada region (Northeastern Morocco) are presented in this study: *Inocybe squarrosa* and *I. rufuloides*. These species are described for the first time in Morocco. The results contribute to better knowledge of the Basidiomycetes diversity in Morocco, especially in the Northeastern part of the country.

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