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Researches on Russulaceous Mushrooms-An Appraisal

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ABSTRACT

***Russulaceae* is one among the large families of the basidiomycetous fungi. Some significant studies during the last decade on their systematics and molecular phylogeny resulted in splitting of well known milkcap genus *Lactarius* s.l. and inclusion of number of gastroid and resupinate members under its circumscription. Presently, there are seven genera (including agaricoid, gasteroid and resupinate members) in this family *viz. Russula* Pers., *Lactarius* Pers., *Lactifluus* (Pers.) Roussel, *Cystangium* Singer & A.H. Smith, *Multifurca* Buyck & Hofst., *Boidinia* Stalper & Hjortstam and *Pseudoxenasma* K.H. Larss. & Hjortstam spread over 1248 + recognised species the world over. Out of a total of about 259 + species/taxa of Russulacous mushrooms,146 taxa of *Russula*, 83 taxa of *Lactarius*, 27 taxa of *Lactifluus*, 2 species of *Boidinia* and 1 species of *Multifurca* are documented from India. In this manuscript an appraisal of the work done on various aspects of the members of the family *Russulaceae* including their taxonomic, molecular, phylogenetic, scanning electron microscopic, ectomycorrhizal, nutritional and nutraceutical aspects has been attempted.**

Keywords: *Russulaceae*, taxonomy, phylogeny, SEM, ECM, nutritional, nutraceutical, review

INTRODUCTION

The family *Russulaceae* Lotsy is one of the 12 families under order *Russulales* Krisel ex P.M. Kirk, P.F. Cannon & J.C. David (Kirk *et al*., 2008). The family was established by Roze (1876) as Russulariéés (nom. nud.), which was subsequently validated as *Russulaceae* by Lotsy (1907). The members of this family are most diverse, containing a remarkable variety of carpophore forms ranging from resupinate to pleurotoid, pileate and stipitate with lamellate or gasteroid hymenophore. Four distinct phylogenetic clades are identified within *Russulaceae* by molecular phylogenetic analysis of ribosomal gene (nrITS and nrLSU) and part of a protein-coding gene (RPB2) (Buyck *et al*., 2008). The vast majority of the known species in the family *Russulaceae* are agaricoid belonging to the traditional genera *Russula* Pers. and *Lactarius* Pers., which are reported to form a monophyletic clade together with their gasteroid and pleurotoid relatives (Miller *et al*., 2006). The bright color of genus *Russula* and latex exuding feature of genus *Lactarius* probably lead Persoon (1797) to name these two genera. *Russula* appeared first as tribe *Russula* Fr. and *Lactarius* appeared as the tribe *Galorrheus* Fr. of the genus *Agaricus* L. as circumscribed by Fries (1821). Later, Gray (1821) elevated the tribe to generic status and both *Russula* and *Lactarius* have maintained a remarkable continuity to the present since then. Multigene based phylogenies of family *Russulaceae* reveals that *Lactarius* and *Russula* are not supported as two monophyletic clades (Buyck *et al*., 2008). It has been documented that the genus *Russula* appears to be monophyletic only when a small group of species representing subsection *Ochricompacta* of genus *Russula* is removed, as these forms an independent clade where *Russula* and *Lactarius* are mixed. A new genus *Multifurca* Buyck & Hofst. was described to accommodate such representatives (Buyck *et al*., 2008). Apart from the taxa that belong to genus *Multifurca*, the genus *Lactarius* also contains two clades, a larger and a smaller one with only 20% taxa (Verbeken et al., 2011). There are 750 species of genus *Russula* and 450 species of genus *Lactarius* documented worldwide (Kirk *et al*., 2008). As compared from India, 146 taxa of *Russula* and 83 taxa of *Lactarius*, 27 taxa of *Lactifluus* (Pers.) Roussel are known. (Atri *et al*., 1994; Das and Sharma, 2005; Bhatt *et al*., 2007; Das 2009; Das *et al*., 2010, 2013, 2015; Das and Verbeken, 2011, 2012; Latha *et al*., 2016; Sharma *et al*., 2016; Paloi *et al*., 2015, 2016; Dutta *et al*., 2015; Khatua *et al*., 2015,Wisitrassameewong *et al*., 2016).

The other genera listed in the family are *Lactifluus*, *Cystangium* Singer & A.H. Smith (Miller *et al*., 2001), *Multifurca* (Buyck *et al*., 2008), *Boidinia* Stalper & Hjortstam and *Pseudoxenasma* K.H. Larss. & Hjortstam (Larsson and Larsson, 2003). The gasteroid genus *Cystangium* is known only from Australia and South America. It shares many characteristics with *Russula* and *Lactarius*. At present, there are 32 species of *Cystangium* known throughout the world (Kirk *et al*., 2008). Genus *Multifurca* is characterized by regularly forked, narrow, orange, pinkish-orange to honey yellow gills, very small spores with a faint, obscurely to distinctly sub-reticulate ornamentation and inamyloid suprahialer spot. Presently only 5 species of this genus are documented worldwide (Kirk *et al*., 2008) and only 1 species *M. roxburghiae* Buyck & Hofst. is known from India. *Boidinia* is a widespread polyphyletic genus represented only by 10 species (Kirk *et al*., 2008). It is a wood inhabiting fungus that strictly forms thin resupinate basidiomata on coniferous wood. Dhingra *et al*. (2012) documented 2 species of *Boidinia*, namely *B. furfuracea* (Bers.) Stalpers & Hjortstam and *B. lacticolor* (Bers.) Hjortstam & Ryvarden from India. *Pseudoxenasma* is another resupinate member represented only by one species, *P. verrucisporum* K.H. Larss. & Hjortstam from Sweden (Kirk *et al*., 2008). It grows on fallen branches and trunks as well as on dead but still attached branches of *Picea* Dietrich. in humid forest. None of the species of *Cystangium* and *Pseudoxenasma* have been documented yet from India. Most of the members of *Russulaceae* including species of *Russula*, *Lactarius* and *Lactifluus* are found closely associated with trees of *Quercus* L., *Lithocarpus* Blume, *Rhododendron* L., *Shorea* Roxb. ex. C.F.Gaertn, *Pinus* L., *Cedrus* Trew., *Abies* Miller, *Picea*, etc. Besides forming symbiotic association, these fungi are also

reported to provide some non-nutritional benefits to tree seedlings. Little work has been undertaken on the characterization of ectomycorrhiza of this particular group of fungi in India.

Some of the members of family *Russulaceae* including *Russula paludosa* Britzelm., *R. xerampelina* (Schaeff.) Fr., *R. lepida* Fr., *Cystangium sessile* (Massee and Rodway) Singer & A.H.Smith are reported to be medicinally important (Wang *et al*., 2007; Lovy *et al.*, 2000; Bougher and Lebel, 2001). Some of the species, including *Russula delica* Fr. and *Lactarius deliciosus* (L.) Gray, are reported to possess antioxidant and antimicrobial properties (Barros *et al*., 2007a, b, c; Türkoğlu *et al*., 2009; Sharma, 2015). Some of the species, including *Lactarius deliciosus* (L.) Gray, *L. sanguifluus* (Paulet) Fr., *L. resimus* (Fr.) Fr., *L. scrobiculatus* (Scop.) Fr., *L. torminosus* (Schaeff.) Pers., *L. piperatus* (L.) Pers., *L. edulis* Buyck, *L. gymnocarpoides* Verbeken, *L. longisporus* Verbeken, *L. xerampelinus* Karhula & Verbeken, *Russula brevipes* Peck., *R. cyanoxantha* (Schaeff.) Fr. and *R. virescens* (Schaeff.) Fr., are most sought after from edibility point of view (Verbeken *et al*., 2000; Atri *et al*., 1997, 2010a,b).

I. TAXONOMIC STUDIES

To begin with, this family was classified under the order *Agaricales* Undrew. (Singer, 1962, 1975, 1986; Smith, 1949, 1973; Pegler 1977; Stuntze, 1977). However, some of the workers including Kühner (1980) and Kreisel (1969) raised the family to the rank of order as *Russulales*. Much earlier Bucholtz (1902) has already attempted to separate this family from order *Agaricales* primarily because of the relationship between two genera of *Russulaceae* and certain hypogeal and epigeal genera of *Hymenogastrales*. Malenćon (1931) also proposed closeness between gasteromycetoid family *Asterogasteraceae* R. Heim and agaricoid family *Russulaceae* and postulated a separate order *Asterosporales* R. Heim for them. Supporting Malenćon (1931), Heim (1938) also placed family *Russulaceae* and family *Elasmomycetaceae* Locq. ex Pegler & T.W.K. Young under the order *Russulales*. Singer (1986) recognized suborder *Russulineae* under order *Agaricales* to include family *Russulaceae* and family *Bondarzewiaceae* Kotlab & Pouzar. The work on the family *Russulaceae* is dominated by the investigations undertaken on two agaric genera, namely *Russula* and *Lactarius*. In view of their close phylogenetic relationship with gasteroid genus *Cystangium*, resupinate genera *Boidinia* and *Pseudoxenasma*, all these have been classified under family *Russulaceae* of order *Russulales* (Miller *et al*., 2001; Larsson and Larsson, 2003). With the addition of yet another closely allied agaricoid genus *Multifurca* by Buyck *et al*. (2008), and splitting of large genus *Lactarius* into *Lactarius* and *Lactifluus* (Buyck *et al.* 2010; Verbeken *et al*. 2011), in all 7 genera spread over 1248 species have been recognized under family *Russulaceae*.

(a) The world scenario: Initially, the taxonomic work on *Russula* and *Lactarius* started simultaneously in Europe and North America. In Europe the earliest work on the genus *Russula* was done by Maire (1910), Peck (1907); Burlingham (1907a,b, 1908, 1910a,b, 1913, 1915, 1918a,b, 1921, 1924, 1932, 1940) and Beardslee (1918). Schaeffer (1952) described 68 species in his monographic work from Europe and provided a key for the identification of 104 taxa of the genus *Russula*. Schaeffer (1952) recognized 3 sections (*Compactae*, *Gratae* and *Ingratae*) and 15 undersections under this genus. Hesler (1960, 1961a, b) described 193 species of *Russula* from North America. Singer (1926) published a monograph on the genus *Russula* which he revised in 1932. Singer (1986) in his treatise "Agaricales in modern taxonomy" recognized 317 species of *Russula* under 11 sections, namely *Pellicularie* Heim., *Delicoarchaeae* Sing., *Plorantes* Bat. ex. Sing., *Crassotunicatae* (Sing.) Sing., *Compactae* Fr., *Pachycystides* Singer, *Metachromaticae* Singer, *Decolorantes* (R. Maire) Singer, *Ingratae* Quél., *Rigidae* Fr. and *Russula*. Romagnesi (1967) was one of the prominent contributors on *Russula* from Europe. He published a detailed monograph containing 190 species and 92 varieties of European and African species of *Russula*. He divided the genus into 9 sections (*Compactae* Fr., *Heterophyllae* Fr., *Ingratae* Quélet, *Piperinae* Quélet, *Incrustatae* Romagn., *Tenellae* Quélet, *Polychromae* R. Maire, *Coccinae* Romagn. and *Insidiosae* Quélet). Pearson (1948) treated 66 species of *Russula* from Britain in 8 sections, namely *Lactariodes*, *Rigidae*, *Resilientes*, *Foetentes*, *Ochraceae*, *Acrae*, *Subacrae* and *Gratae*. Rayner (1968-1970) studied British species of *Russula* and described 112 species of the genus under 2 divisions, namely *Compactae* and *Genuinae*. Antoine (1973) designed a key for 96 species of *Russula* from Eastern France. An outstanding monographic work was published by Sarnari (1998) on the European *Russula*. It deals with the critical analysis of all of the various nomenclature problems and is probably the best currently available classification for the genus. Sarnari (1998) divided the genus into six subgenera, namely *Compactae* (Fr.) Bon., *Ingratula* Romagn., *Amoenula* Sarnari, *Heterophyllidia* Romagn., *Incrustatula* Romagn., *Russula* Romagn. and stressed the importance of microscopic features in *Russula* taxonomy.

The work on the rich African flora of *Russulaceae* particularly on *Russula* has been undertaken by Buyck (1988, 1989 a, b, c, 1990 a, b, 1991, 1993, 1994, 1995, 1997, 1999 a, b). Fatto (1996 a, b, 1998 a, b, 1999, 2000) has also made significant contributions on the genus *Russula* from North America. Kibby and Fatto (1990) published a comprehensive key on the "*Russula*" of North America.

Our knowledge on *Russula* taxonomy has been enhanced as a result of significant contributions by Krauch (1994, 1998, 1999, 2001) from Europe; Thiers (1979) from California; Romagnesi (1936, 1940, 1942, 1943, 1945, 1958, 1967, 1970, 1972, 1996) from France and Pearson (1948) from Europe who gave a brief account of 53 species of *Russula* from Britain. Significant contributions on *Russula* taxonomy have been made by Jurkeit and Krauch (2000, 2001) from Germany, Buyck and Ovrebo (2002) from Panama, and Vesterholt (2002) from Denmark. Some of the latest contribution on *Russula* taxonomy is by Adamčik (1998, 2001, 2002, 2004); Adamčik and Knudsen (2004); Adamčik *et al.* (2003, 2006); Manassila *et al*. 2005; Ronikier (2005, 2009); Ronikier and Adamčik (2009a, b); Miller *et al.*

(2006); Buyck *et al*. (2006); Nakamori and Suzuki (2007), etc. Adamčik *et al*. (2010) described *R. ochrifloridana* Buyck & Adamčik, a new species of *Russula* from Florida. There are some recent publications on *Russula* taxonomy by Adamčik and Buyck (2011, 2012); Buyck and Adamčik (2011); Buyck and Atri (2011); Caboň and Adamčik (2011); Caboň *et al*. (2013) and Adamčik *et al*. (2013).

Based on the recent phylogenetic advancement, the genus *Russula* has been divided into nine subgenera: *R.* subg. *Compactae*, *R*. subg. *Heterophyllidia*, *R.* subg. *Ingratula*, *R*. subg. *Amoenula*, *R*. subg. *Incrustatula* and *R*. subg. *Russula*, *R.* subg. *Archaea*, *R.* subg. *Brevipes* and *R.* subg. *Malodora* (Sarnari, 1998; Hongsanan, 2015).

As has been the case on *Russula* taxonomy, most of the classical work on *Lactarius* systematics has also come from Europe and North America. In Europe monographic study on *Lactarius* was initiated by Knauth and Neuhoff (1937). Neuhoff (1956) while working on the genus described 69 species in his survey of European flora. In North America, the pioneer work on *Lactarius* was done by Peck (1872, 1884). Detailed monographic work on genus *Lactarius* from North-America has been attempted by Hesler and Smith (1979). In this monograph, the genus has been divided into 6 subgenera [*Lactarius* Hesler & Smith, *Plinthogalus* (Burl.) Hesler & Smith, *Lactifluus* (Burl.) Hesler & Smith, *Piperites* (Fr.) Kauffman, *Tristes* Hesler & Smith and *Russularia* (Fr.) Kauffman], 18 sections and 5 subsections. As many as 200 species and 60 varieties of genus *Lactarius* s.l. are keyed out and described in this monograph. Yet another outstanding contribution on the genus *Lactarius* is by Heilmann-clausen *et al*. (1998) in which the genus has been divided into 6 subgenera, namely *Lactarius* Pers., *Lactifluus* (Burl.) Hesler & A.H. Smith, *Lactariopsis* (Henn.) R. Heim., *Piperites* (Fr.) Kauffman, *Russularia* (Burl.) Kauffman and *Plinthogali* (Burl.) Hesler & A.H.Smith. In Europe the taxonomy of *Lactarius* has been extensively investigated by Basso (1994, 1997, 1998, 1999a, b, 2000, 2001) and Basso *et al*. (2001). From Mexico Montoya *et al*. (1990, 1996, 1998 and 2003) and Montoya and Bandala (2003, 2004a, b, 2008) significantly contributed on the *Lactarius* taxonomy. Yet another significant contribution on the *Lactarius* flora of Africa and South East Asian tropical Rain forest has come from the work of Verbeken (1995a, b, 1996a-d). While working on this genus in Africa Verbeken (1998a) documented 23 species of *Lactarius* and also emended the subgenus *Lactifluus* of the genus *Lactarius* by excluding the species representing temperate sections *Albati* (Bat.) Singer and *Allardii* Hesler & Smith from the circumscription of subgenus *Lactifluus* and subsequently Verbeken (1998b) proposed to include the temperate section *Albati* (Bat.) Singer emended by Hesler and A.H. Smith (1979) in subgenus *Lactariopsis* (Henn.) R. Heim. Besides the relationships of the section *Chamaeleontini* Sing. with *Russula* subsection *Amoeninae* Singer ex Buyck and subgenus *Lactariopsis* (Henn.) R. Heim.with the genus *Pleurogala* Redhead & Norvell were also discussed. Verbeken and Horak (1999, 2000) proposed 23 new taxa for the genus *Lactarius* besides three previously described species. Verbeken *et al*. (2000) recorded 35 species of *Lactarius* from relatively xeric Zambezian miombo woodlands in Zimbabwae. Verbeken (2000, 2001); Nuytinck *et al*. (2004) and Eberhardt and Verbeken (2004) studied *Lactarius* species from Europe and Tropical Africa. Van de Putte *et al*., 2010 described *Lactarius* from Northern Thialand. Wang *et al*. (2006) described *Lactarius ochrogalactus* Hashiya, a new species of genus *Lactarius*. Škubla (2006) proposed *Lactarius hrdovensis* Škubla a new species from Slovakia. The other important contributions on the genus are by Wang (2007), Nuytinck and Verbeken (2003, 2007); Huyen *et al*. 2007 and Nuytink *et al*. (2004, 2006, 2007). Recently based upon the multigene phylogenetic evaluation Buyck *et al*. (2010) and Verbeken *et al*. (2011) proposed the carving out of a new genus *Lactifluus* (Pers.) Roussel out of larger genus *Lactarius*. In this preposition the genus name *Lactarius* has been conserved for the larger clade representing the subgenera *Piperites*, *Russularia* and *Plinthogalus* while in the smaller clade the new genus *Lactifluus*, which represent about only 20% species of the larger genus *Lactarius* has been classified with species representing the former subgenera *Lactarius*, *Lactariopsis*, *Russulopsis*, *Lactifluus* and *Gerardii* and the former section of subgenus *Lactarius* sect. *Edules*. The proposal of Buyck *et al*. (2010) in this regard also got the support of Nomenclature Committee for fungi (Norvell, 2011) which was subsequently approved by the General Committee and accepted by the 2011 International Botanical Congress (Barrie, 2011; McNeill *et al*., 2011). Verbeken *et al*. (2011) described number of new combinations in genus *Lactifluus*, which basically are the species of *Lactarius* in the older concept described under subgenera *Edules*, *Lactariopsis* and *Russulopsis*. Similarly Stubbe *et al*. (2010, 2012 a, b) described new combinations in *Lactifluus*, *L*. subg. *Gerardii* (A.H. Smith & Hesler) Stubbe. Verbeken *et al*. (2012) described this in *L.* subg. *Lactifluus* and *Piperati*. Miller *et al*. (2012) proposed a new species of *Lactifluus* i.e. *Lf. subiculatus* S.L. Mill., Aime & T.W. Henkel and 3 species of *Russula* i.e. *R. myremecobroma* S.L. Mill., Aime & T.W. Henkel, *R. paxilliformis* S.L. Mill., Aime & T.W. Henkel and *R. gelatinivelata* S.L. Mill., Aime & T.W. Henkel from Guyana. *Lactifluus kigomaensis* De Crop & Verbeken, a new species was documented from Tanzania by De Crop *et al*. (2012). New species of *Lactifluus* i.e. *Lf. dunensis* Sá & Wartchow and *Lf. aurantiorugosus* Sá & Wartchow has been proposed by Mariana *et al*. (2013) and Mariana and Wartchow (2013) from Brazil. De Crop *et al*. (2014) studied the allied species of *Lactifluus piperatus* from Western Europe. Verbeken *et al*. (2014) described some angiocarpous representatives of *Russulaceae* from South East Asia. Other recent contributors are by Basso *et al*. (2001); Nuytinck and Ammirati (2014); Maba *et al*. (2014, 2015); Wisitrassameewang *et al*. (2014a, b, 2015); Wang *et al*. (2015a); Van de Putte *et al*. (2016).

Gasteroid genus *Cystangium*, which is a part and parcel of family *Russulaceae* in the newer dispensation (Kirk *et al*., 2008) has been investigated by Lebel and Trappe (2000), Lebel and Castellano (2002) and Trappe *et al*. (2002). As many as 11 new species of *Cystangium* were described by Lebel (2003) from Australia. *Multifurca* is yet another russuloid new genus described by Buyck *et al*. (2008). Only 5

species of this genus are reported worldwide (Kirk *et al.*, 2008). *Boidinia* and *Pseudoxenasma* are resupinate members of family *Russulaceae*. Various species of *Boidinia* have been described by Ginns and Freeman (1994) and Wu and Buchanan (1998). Larsson (2007) included this genus in family *Russulaceae* on the basis of phylogeny. *Pseodoxenasma,* another resupinate genus is represented by only one species described by Larsson and Hjortstam (1976). On the basis of phylogeny Larsson and Larsson (2003) described its affinities with *Russulales*.

(b). The Indian scenario: The earliest references of documentation of russulaceous mushrooms from India are those of Berkeley (1851, 1852, 1854, and 1876) who reported 8 species of *Russula* i.e. *R. alutacea* Fr., *R. cinnabarina* Hook, *R. emetica* Fr., *R. furcata* Fr., *R. grossa* Berk., *R. lepida* Fr., *R. rubra* (Fr.) Fr. and *R. sanguinea* Fr. Out of these *R. alutacea* was reported from Kashmir and rest from Eastern Himalayas and 5 species of *Lactarius* i.e. *L. deliciosus* (L.) Gray, *L. princeps* Berk., *L. stramineus* Berk., *L. subdulcis* (Pers.) Gray and *L. vellereus* (Fr.) Fr. along with number of other mushrooms. Chona *et al.* (1958) recorded *Lactarius cilicioides* (Fr.) Fr. from Delhi.

Major contributions from Eastern Himalayas are by Ray and Samajpati (1980), Shajahan and Samajpati (1995) and Das (2009, 2010). Das *et al.*, 2010 reported 2 new species of *Russula* i.e. *R. khanchanjungae* K. Das, Van de Putte & Buyck and *R. tsokae* K. Das, Van de Putte & Buyck from subtropical to temperate forest of Sikkim Himalayas. They also described *R. griseocarnosa* Wang, Yang & Knudsen for the first time from India which was earlier reported from China. Das and Verbeken (2011, 2012), described 5 new species of *Lactarius* i.e. *L. elaiviscidus* Das & Verbeken, *L. ermineus* Das & Verbeken, *L. byssaceus* Das & Verbeken, *L. crenulatus* K. Das & Verbeken, *L. croceigalus* K. Das & Verbeken and new records of *Lactifluus* subg. *Gerardii* from Sikkim. Das *et al*. (2013) described three new species of *Russula* i.e. *R. sharmae* Das, Atri & Buyck, *R. dubdiana* Das, Atri & Buyck and *R. sikkimensis* Das, Atri & Buyck from west district of Sikkim. Five novel species of *Lactarius* namely, *Lactarius vesterholtii* K. Das & D. Chakr., *L. olivaceoglutinus* K. Das & Verbeken, *L. pyriodorus* K. Das & Verbeken, *L. yumthangensis* K. Das & Verbeken and *L. indochrysorrheus* K. Das & Verbeken were also recently described from North Sikkim (Das and Chakraborty 2014, Das *et al.*, 2015). Similarly, three new species of the sister genus *Lactifluus* namely, *Lf. dissitus* Van de Putte, K. Das & Verbeken, *Lf. leptomerus* Van de Putte, K. Das & Verbeken and *Lf. versiformis* Van de Putte, K. Das & Verbeken (Van de Putte *et al*. 2012) was also described from this part of Himalaya.

Ramakrishnan *et al*. (1952), documented *Russula lepida* Fr. from Tamil Nadu. Other contributions from Maharashtra, Tamil Nadu and Kerala are by Patil and Thite (1978); Natarajan (1978); Sathe and Daniel (1980). Natarajan and Raaman (1983) described *R. parazurea* Schaeff. from Madras. Vrinda *et al.* (1997) proposed a new species of *Russula* i.e. *R. leelavathyi* Vrinda, Pradeep & Abraham from South India. Recent contributors from south India are by Pradeep and Vrinda (2007); Mohanan (2011); Farook *et al*. (2013) and Latha *et al.* (2016).

Pioneer work from North West Himalayas is by Mundkar (1938) who reported *R. rosacea* from Mussoorie hills. Bakshi (1974) reported mycorrhizal association of some russulaceous mushrooms. Sharma *et al*. (1978) and Kumar *et al*. (1979) documented some fleshy fungi from Himachal Pradesh. Watling and Gregory (1980) described 11 taxa of *Russula* i.e. *R. brevipes* Peck., *R. densifolia* (Secr.) Gill., *R. firmula* Schaeff., *R. fragrantissima* Romagn., *R. nauseosa* var. *nauseosa* (Pers. ex. Secr.) Fr., *R. nauseosa* var. *atropurpurea* (All.) Sing., *R. nauseosa* var. *xanthophaea* (Boud.) Singer, *R. persicina* Krombh., *R. rubricunda* Quél. and *R. sanguinea* (Bull.) Fr. from Kashmir. Abraham *et al*. (1980, 1981) reported some species of *Russula* and *Lactarius* from Kashmir. Major taxonomic contributions particular to this family are by Saini and Atri (1981, 1982a, b, 1984, 1989a, b, 1990, 1993, 1999); Atri 1985; Saini *et al*. (1982, 1988, 1989, 1993); Atri and Saini (1986, 1988, 1989, 1990a, b, c); Atri *et al*. (1991a, b, c, 1992, 1993a, b, 1994, 1997); Kaur (1995). They described 53 taxa of *Russula* and 48 taxa of *Lactarius* from North West Himalayas. Sharma (2015) investigated 63 taxa of russulaceous fungi taxonomically, out of which 32 belong to genus *Russula*, 23 to *Lactarius* and 8 species to genus *Lactifluus*. Rawla and Sarwal (1983) described 3 new species of *Russula* i.e. *R. mussoriensis* Sarwal, *R. himalayana* Sarwal, *R. schaefferina* Sarwal and 2 new records *R. pectinata* Fr. and *R. odorata* Romagn. from North West Himalayas. Other workers who documented the diversity of this family are Manjula (1983); Sarwal (1984); Abraham and Kaul (1985); Bhatt *et al.* (1995, 1999, 2000, 2007); Bhatt and Lakhanpal (1988a, b, c, 1990); Rawla (1994, 2001, 2002); Natarajan *et al*. (2005); Manimohan and Latha (2011). Extensive work on this family, from the Kumaon region of Uttarakhand has been done by Das *et al*. (2002, 2003, 2004a, b, 2005a, b, c, 2006a, b, 2015); Das and Sharma (2001a, b, 2002, 2003, 2004a, b, 2005); Sharma and Das (2002, 2003) and Sharma *et al.* (2004). Other recent workers, who have contributed towards the diversity of this family are Riviere *et al.* (2006, 2007); Dar *et al*. (2009); Saini *et al*. (2010) and Sharma *et al*. (2012, 2016). Dhancholia (2011) worked on family *Russulaceae* in Lahaul valley, Himachal Pradesh. Buyck and Atri (2011) reported a *Russula* species with unprecedented hymenophore configuration. Wisitrassameewong *et al*. (2016) described five species of *Lactarius*, namely *L. atrii* Van de Putte & K. Das, *L. dombangensis* Verbeken & Van de Putte, *L. flavigalactus* Verbeken & K. Das, *L. lachungen* Verbeken & Van de Putte and *L. sikkimensis* Verbeken & K. Das as new to science. No record of gasteroid genus *Cystangium* is there from India. Only 1 species of genus *Multifurca* has been described from India based upon collection described by Saini and Atri (1982a) as *Russula grossa* on an earlier occasion. Dhingra *et al*., 2012 documented 2 species of *Boidinia*, namely *B. furfuracea* (Bers.) Stalpers & Hjortstam and *B. lacticolor* (Bers.) Hjortstam & Ryvarden from India. No record of resupinate member *Pseudoxenasma* is there in the Indian documentation. From India, so far the family *Russulaceae* is represented by about 259 + species/ taxa (146 of *Russula*,

83 of *Lactarius*, 27 of *Lactifluus*, 2 species of *Boidinia* and 1 species of *Multifurca*) as compared to 1248 + species of these genera reported the world over.

II. SEM STUDIES

Spore ornamentation is one of the important taxonomic characters for distinguishing species within russulaceous fungi. In 1924, Melzer for the first time discussed the importance of spore ornamentation in *Russula*. Malenćon (1931) suggested that it is the outer wall which is torn into fragments and form warts and bands during spore growth. Josserand (1940) gave a view that the ornamentation pattern on the spore surface is a molded structure inherent in spore itself. Moreau (1930) and Pearson (1950) used light microscopy to study the spore ornamentation in these mushrooms. Spore ornamentation in *Lactarius* was studied by Heim (1955). Later Perreau and Heim (1969), Moore and Grand (1970) and Grand and Moore (1970) used SEM to study spore morphology. Hesler and Smith (1979) studied the spore ornamentation in 59 taxa of North American *Lactarius* and described 5 basic types of ornamentation on the basis SEM i.e. 1) Tuberculate , 2) Rugose , 3) Incomplete reticulation, 4) Complete reticulation and 5) Zebroid . Das *et al.* (2004 a, b) also studied the ornamentation of spores in 4 species of *Lactarius* i.e. *L. capitatus* Das, Sharma & Montoya, *L. sanjappae* Das, Sharma & Montoya, *L. mukteshwaricus* Das, Sharma & Montoya and *L. verbekenae* Das, Sharma & Montoya. Recently Gussema *et al.* (2005) studied the Raman Spectra in 4 species of *Lactarius* i.e. *L. controversus* Pers.:Fr., *L. lacunarum* (Romagn.) ex Hora, *L. quieticolor* Romagn. and *L. quietus* Fr. They observed high amount of lipids and oleate as the main fatty acid. Atri *et al.* (2014) investigated the SEM details of 51 taxa of Russulaceous mushrooms from North West Himalayas belonging to *Russula*, *Lactarius*, and *Lactifluus* and documented eight types of exosporial ornamentations viz., Tuberculate, Catenulate, Winged type-A , Winged type-B, Incomplete reticulate, Complete reticulate , Ridged and Rugulose type.

III. MOLECULAR STUDIES

During the past decade molecular tools were employed to investigate the intricate taxonomic problems and molecular phylogenetic analysis has been attempted in agaricoid, pleurotoid, secotioid and gasteroid *Russulaceae* by number of investigators including Bruns *et al*. (1998); Martin *et al*. (1999); Moncalvo *et al.* (2000); Calonge and Martin (2000); Henkel *et al.* (2000); Miller *et al.* (2001); Peter *et al.* (2001b); Nuytinck *et al*. (2004); etc. Worldwide phylogeny of *Lactarius* section *Deliciosi* inferred from ITS and glyceraldehydes 3- phosphate dehydrogenase gene sequences was investigated by Nuytinck *et al.* (2007) and Nuytinck and Verbeken (2007). Shimono *et al*. (2007) studied molecular phylogeny of *Lactarius volemus* (Fr.: Fr.) Kuntze from the LSU rDNA. Huyen *et al*. (2007) and Buyck *et al*. (2008) carried out molecular phylogenetic analyses of russulaceous mushrooms using ribosomal gene (ITS-nuclear LSU) and a part of protein-coding gene (RPB2) which indicated that four phylogenetically distinct clades could be identified within the family *Russulaceae*. Stubbe *et al*. (2010) studied delimitation within *Lactarius gerardii* species complex. Van de Putte *et al*. (2009, 2010) studied phylogenetic aspects of genus *Lactarius*. Phylogenetic analysis of wild edible *Russula* on the basis of ITS sequence was done by Manassila *et al.* (2005) from North East Thailand. Upadhyay *et al*. (2010) using RAPD and isozyme analysis studied genetic and biochemical diversity of *Russula* species. On the basis of molecular studies and phylogenetic analyses Buyck *et al*. (2010) and Verbeken *et al*. (2011) carved a new genus *Lactifluus* out of the larger genus *Lactarius*. Van de Putte *et al*. (2016) investigated *Lactifluus volemus* in Europe by adopting a multilocus genealogical approach.

IV. ECTOMYCORRHIZAL STUDIES

There is substantial information available about the ectomycorrhizal fungi and their role in the growth and development of associated plants. Already over 400 taxa of ectomycorrhizal fungi, mostly belonging to *Russulaceae* Lotsy, *Boletaceae* Chevall., *Cantherallaceae* J. Schröt, *Amanitaceae* R. Heim ex Pouzar and *Cortinariaceae* R. Heim ex Pouzar have been described from African forest and woodland and similar number is likely to be reported in future (Castellano *et al*., 2000). They are the dominating mycorrhizal symbionts in boreal, temperate and some tropical forests. Literature survey revealed that most ectomycorrhizal fungi are basidiomycetes, with *Amanita* Pers., *Cortinarius* (Pers.) Gray, *Lactarius, Russula* and *Suillus* Gray among the best known ectomycorrhizal genera (Hacskaylo, 1972). Ectomycorrhizal associations are widespread, particularly in temperate regions, and involve many of the ecologically important trees making up the forests characteristic of these regions. Prominent examples include deodar, hemlock, spruce, pine, fir, willow, oak, birch, and beech. Besides forming symbiotic association, the fungi also provide some non- nutritional benefits to tree seedlings such as ameliorating the effect of toxic heavy metals (Wilkinson, 1991; Wilkinson and Dickinson, 1995), reducing host water deficit under condition of mild drought (Parkee *et al.*, 1983) and protecting seedling from infection by soil born root pathogens (Chakravarty and Unestam, 1987).

The taxonomic diversity of ECM symbiont is very large as number of fungal symbionts and plant species are reported to be capable of forming ECM associations (Das 2009, 2010; Taylor and Alexander, 2005). Agerer (1987-2002) recognized two main types of hyphal development within ECM mantle types i.e. pseudoparenchymatous and plectenchymatous which have been used in taxonomic characterization of fungal symboints (Agerer, 1987-2002; Agerer *et al*., 1994, 1996-2004). Many ECM fungi are reported to form mantles that are hydrophobic and even hydrophilic (Agerer, 1987-2002). Read and Perez-Moreno (2003) and several recent studies have utilized molecular markers to localize the mycelium of ECM fungal species in different soil layers and substrates. (Dickie *et al*., 2003; Guidot *et al*., 2003; Landeweert *et al*., 2003; Koide *et al.*, 2005). EMF host specificity has been well documented in several genera by number of workers including Den Bakker *et al*., (2004); Moreau *et al.*, (2006); Nouhra *et al.*, (2008); Montecchi and Sarasini (2000); Diez *et al.*, (2002); Bruns *et al*., (1998, 2002), etc.. It is also well

established that some individual EMF species have strong host preferences (Molina, 1979; Murat *et al.*, 2004; Lian *et al.*, 2006; Yuan *et al*., 2010). Highly diverse EMF communities dominated by rare taxa have been documented from a variety of habitats and geographic locations (Taylor, 2002; Gardes and Bruns, 1996; Gehring *et al*., 1998). EMF communities associated with oak trees are reported to be particularly rich (Morris *et al*., 2008; Walker *et al*., 2005). Spatially, many investigators have observed that there is considerable small scale EMF species turnover spanning only 1 to 4 meters, or between trees (Gehring *et al*., 1998; Jonsson *et al*., 2000; Peter *et al*., 2001a, b; Tedersoo *et al*., 2003; Lilleskov *et al*., 2004) among years, but with overall stability in the dominant species on a larger scale, such as across a sampling site (Izzo *et al*., 2005). Agerer (1986, 1991, 1995); Beenken (2001, 2004); Lee *et al*. (1997) and Niazi *et al*. (2006) studied the morphological and anatomical ectomycorrhizal details of genus *Russula* and *Lactarius*. Becerra *et al*. (2005) studied the anatomical and molecular ectomycorrhizal characterization of *Lactarius* and *Russula* on *Alnus acuminata*. Other recent workers who studied the mycorrhizal association of *Russula* and *Lactarius* are Toju *et al*., (2013) and Wang *et al*., (2015b). Very recently, phylogenetic studies have been conducted on *Russulaceae* associated with mycoheterotroph *Monotropa uniflora* (*Ericaceae*) from Mexico (Kong *et al*. 2015).

In India, Bakshi (1974) reported some larger fungi forming mycorrhizal associations with forest trees. Natarajan (1987) documented a number of mycorrhizal fungi from southern India associated with *Pinus patula* Schiede ex Schlechtendal et Chamisso and reported *in vitro* synthesis of mycorrhizae of this tree species. Lakhanpal (1989) presented an account of the systematics and ectomycorrhizal relationships of some mushroom families in the North West Himalayas. Sharma and Singh (1990) reported a number of ectomycorrhizal fungi associated with different forest trees of Himachal Pradesh. There are few preliminary studies in the Himalayan region, dealing with the symbiosis rather than the function of ectomycorrhizae in the forest ecosystem (Kumar *et al*., 1990; Dubey *et al*., 1998; Ginwal, 1994). Recent researches conducted in glass-house experiments from Indian Central Himalaya also showed significant increase in root: shoot ratio and plant biomass in the seedling inoculated with mycorrhiza as compared to uninfected once (Pande, 2003; Aggarwal, 2007). Russulaceous fungi include a very rich percentage of ectomycorrhizal species serving the obligate requirement of the forest trees besides few edible and medicinally important species. Most of the members of *Russulaceae* are found closely associated with forest trees such as *Quercus* L., *Rhododendron* L., *Shorea* Roxb. ex. C.F.Gaertn, *Pinus* L., *Cedrus* Trew., *Abies* Miller, *Picea* Dietrich., etc. Saini and Atri (1984) listed 24 species of *Russula* and 5 species of *Lactarius* associated with deciduous, coniferous, and mixed forests in North West Himalayas. Atri and Saini (1986) in further contributions gave an account of the association of 37 taxa of *Russulaceae* (25 taxa of *Russula* and 12 taxa of *Lactarius*) with the gymnospermous and angiospermous trees in North West Himalayas. Atri *et al*. (1997) discussed the distribution and ecology of genus *Russula* in North western Himalayas along with association of its species with Oak, Deodar and Fir in this phytogeographic region. Kumar and Atri (2016) investigated the ectomycoorhizal association of *Russula feugiana* Singer and *Lactifluus volemus* var. *volemus* (Fr.:Fr.) Kuntze with *Shorea robusta* Gaertn growing in Indian Shivaliks.

V. NUTRITIONAL AND NEUTRACEUTICAL STUDIES

Like other edible mushrooms, several members of russulaceous fungi such as *Lactarius deliciosus* (L.) Gray, *L. sanguifluus* (Paulet) Fr., *L. resimus* (Fr.) Fr., *L. scrobiculatus* (Scop.) Fr., *L. torminosus* (Schaeff.) Pers., *L. piperatus* (L.) Pers., *L. edulis* Buyck, *L. gymnocarpoides* Verbeken, *L. longisporus* Verbeken and *L. xerampelinus* Karhula & Verbeken are reported to be edible (Verbeken *et al*., 2000). Amongst the Indian russulaceous mushrooms *R. cyanoxantha* (Schaeff.) Fr., *R. virescens* (Schaeff.) Fr. and *Lactarius deliciosus* (L.) Gray are most sought after species from edibility point of view (Atri *et al*., 1997; 2010 a, b). *Russula emetica* (Schaeff.) Pers. is another mushroom which is although acrid to taste but it is reported that it can be dried and powdered to make a chilli pepper substitute (Arora, 1986). *Russula xerampelina* (Schaeff.) Fr. is one of the few mushrooms with a flavor to compliment green olives. Many workers including Cağlarlrmak *et al*. (2002); Cağlarlrmak (2007); Agrahar-Murugkar and Subbulakshmi (2005); Barros *et al*. (2007a, b, c, d); Pushpa and Purushothma (2010); Ayaz *et al*. (2011); Khatua *et al*. (2015); Onbasili *et al*., (2015); Teklit (2015); Kouassi *et al*., (2016) and Toshinungla *et al*. (2016) worked on the nutritional aspects of *Russula* and *Lactarius*. Riberio *et al*. (2008) evaluated the mean content of carboxylic acid in various mushrooms including *R. cyanoxantha*.

A pepide extracted from *Russula paludosa* Britzelm. has been reported to show HIV-1 reverse transcriptase inhibitory activity (Wang *et al*., 2007). *Russula xerampelina* extract was shown to be inhibitory to the growth of *Plasmodium falciparum* Welch., a pyrimethamine resistant malarial parasite (Lovy *et al.*, 2000). Antioxidant and antimicrobial activity of *R. delica* Fr. have been studied by number of workers including Türkoğlu *et al*. (2009); Barros *et al*. (2007a, 2008a, b, c) and Gursoy *et al*. (2010). Barros *et al*. (2007c) reported high antioxidant activity among widely consumed *Cantharellus cibarius* Fr., *Lactarius deliciosus* (L.) Gray and *Agaricus arvensis* Schaeff. Antitumor activity shown by the 'lepida acid A' from *Russula lepida* Fr. was studied by Jian-Wen *et al*. (2000). Lactaroviolin isolated from *L. deliciosus* has been reported to possess antimicrobial (antibiotic) activity against *Mycobacterium tuberculosae* Zopf. (Barros *et al*., 2007b). Similarly *Lactarius chrysorrheus* Fr. has been reported to possess antibiotic activity against gram -ve bacteria, such as *Escherichia coli* Castellani & Chalmers and *Shigella sonnei* (Levine) Weldin. *Cystangium sessile* (Massee and Rodway) Singer & A.H. Smith is also reported to possess medicinal properties (Bougher and Lebel, 2001). Sharma (2015) evaluated 7 edible species of russulaceous mushrooms, namely *Russula brevipes*, *R. cyanoxantha*, *R. heterophylla*, *R. virescens*, *Lactarius deliciosus*, *L. sanguifluus* and *Lactifluus piperatus*

for their nutritional and neutraceutical profile. Nutritional analysis confirmed the presence of good amount of proteins (19.84-37.77%), carbohydrate (40.81-63.24%), fat (1.7-5.44%) , ash (6.17-16.43%), moisture (6.89-8.34%) and energy value ranging between 253.84-287.40 kcal/100g of the sample. Mannitol and trehalose were the main sugars documented. Amongst the nutaceutical components, Sharma (2015) documented phenolic content (1.78-17.55mg/g), flavonoid (0.14-2.47 mg/g), ascorbic acid (0.12-0.31 mg/g) and β carotene (4.47-32.73 µg/g). When tested, these mushrooms are reported to exhibit antioxidant properties. Maximum reducing power was exhibited by *Lactarius sanguifluus* followed by *Russula cyanoxantha*, *Lactarius deliciosus*, *Russula heterophylla*, *R. virescens*, *Lactifluus piperatus* and *Russula brevipes* which gave minimum reducing activity.

CONCLUSION

There is a substantial gap between what we really know and what actually exist in nature as far as mushroom mycobiota is concerned. Therefore it is an exigency to undertake extensive and intensive explorations, inventorize through a thorough morphotaxonomy followed by establishing phylogenetic relationships and evaluate them for their nutritional and nutraceutical attributes including their importance in the establishment of mycorrhiza for the long term sustainance of forest ecosystem. Edible russulaceous mushrooms compare well with the nutritional and neutraceutical qualities of commonly cultivated mushrooms and are much better because of low fat and the presence of neutraceutically important constituents from many of the commonly consumed vegetables and meat. Raising semicultures of edible mycorrhizal russulaceous mushrooms with their respective mycorrhizal hosts is a viable option for their conservation and subsequent propagation in their natural habitats.

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