



IJABBR- 2014- eISSN: 2322-4827

International Journal of Advanced Biological and Biomedical Research

Journal homepage: www.ijabbr.com



Original Article

Variability in Agronomic and Chemical Characteristics of Spearmint (*Mentha spicata* L.) Genotypes in Ethiopia

Beemnet Mengesha Kassahun*¹, Desta Fikadu Egata¹, Tewodros Lulseged², Wondu Bekele Yosef¹, Seferu Tadesse¹

¹Ethiopian Institute of Agricultural Research (EIAR), Wondo Genet Agricultural Research Center. P. O. Box 198, Shashemene, Ethiopia

²Ethiopian Institute of Agricultural Research (EIAR), Debre Zeit Agricultural Research Center P.O.Box 32, Debre Zeit, Ethiopia

ARTICLE INFO

Article history:

Received: 02 August, 2014

Revised: 26 August, 2014

Accepted: 18 September, 2014

ePublished: 30 October, 2014

Key words:

Essential Oil

Ethiopia

Spearmint

ABSTRACT

Objective: Evaluation activity was conducted using three spearmint genotypes (WG-SPM-Engl, SPM-Fran and SPM-EAS) and one standard check (WGSM-03) at Wondo Genet, Awassa, Debre Zeit and Allagae during 2012 and 2013 for two years. **Methods:** The treatments were arranged in randomized complete block design with four replications. Data on leaf to stem ratio, leaf yield/ha, Essential oil content and essential oil yield were collected and statistically tested. The combined analysis of variance over the testing locations and years indicated the existence of significant variation among spearmint genotypes for all the characters considered in this study. **Results:** The respective leaf to stem ratio, leaf yield/ha, essential oil content and essential oil yield of the tested genotypes ranged from 1.29 to 2.2, 10.82 to 13.45 t, 0.16 to 0.5% and 18.13 to 67.99 kg, respectively. The highest leaf to stem ratio (2.2) was demonstrated by WG-SPM-Engl and the lowest (1.29) by WG-SPM-Fran. The highest leaf yield/ha was demonstrated by WG-SPM-Fran followed by WG-SPM-Engl; while the lowest value for these agronomic characters were produced by WG-SM03. Compared with WGSM 03, WG-SPM-Fran demonstrated a respective percent increase value of 47.05 and 81.45% on essential oil content and essential oil yield. The overall highest value for essential oil content (0.39%) was recorded at Allagae followed by Wondo Genet (0.35%) and Hawassa (0.34%); while the least value was recorded at Debre Zeit (0.31%). Averaged over the testing locations, the highest essential oil yield/ha (48.29 kg) was recorded at Hawassa and the lowest at Wondo Genet (34.85 kg). Compared with second testing year, a respective percent increase value of 12.12 and 23.18% on essential oil content and essential oil yield was demonstrated in first testing year.

1. INTRODUCTION

Mints comprise a group of species of the genus *Mentha* belonging to the family Lamiaceae (Edris *et al.*, 2003). Among mint species, spearmint (*Mentha spicata* L.) is considered industrial crop as it is a source of essential

oils enriched in certain monoterpenes like carvol, dihydrocarveole, dihydrocarveylacetate, menthol, menthone, caryophyllene, terpineol and cubebene which is widely used in food (Edris *et al.*, 2003), flavor (El-Wahab and Mohamed, 2009), cosmetic and pharmaceutical industries (Foda *et al.*, 2010).

*Corresponding Author: Beemnet Mengesha Kassahun, Ethiopian Institute of Agricultural Research (EIAR), Wondo Genet Agricultural Research Center. P. O. Box 198, Shashemene, Ethiopia (mengeshabeemnek@gmail.com)

Spearmint is well adapted to climatic conditions in tropical and subtropical areas. It can be cultivated in wide range of soils and found in back gardens of homesteads. A climate with adequate and regular rainfall and good sunshine during its growing period ensures a good yield (Shormin *et al.*, 2009). Mint in general is succulent crop that has a high water requirement during its active growth period. The water requirements of mints differ from location to location depending on soil type, soil fertility status and climatic factors. Both high soil moisture content and high soil moisture tension (water stress) decrease the growth, herb and essential oil yields of mints (Shormin *et al.*, 2009). In addition, the yield and the essential oil composition of mint species are influenced by interaction between the genotype and environment, method of distillation, kind of storage, crop age, time of harvest and season (Chalchat *et al.*, 1997; Farooqi *et al.*, 1993; Gasic, 1989), plant management and harvesting age (Solomon and Beemnet, 2011). As illustrated by (Verma *et al.*, 2010), the time of harvest, in general has a close relation to yield and quality of oil and it varies from place to place and genotype to genotype. Currently, Wondo Genet Agricultural Research Center is doing a number of awareness creation activities about the production, processing, marketing and utilization of aromatic and medicinal plants at national level. As an outcome, the number of entrepreneurs who wants to cultivate aromatic and medicinal plants is becoming increasing time to time in the country. Hence, it is a prerequisite to evaluate the performance of spearmint under different locations of Ethiopia for its agronomic and chemical characters for getting optimum benefit out of its cultivation. Therefore, the primary objective of this

activity was designed to evaluate the performance of spearmint genotypes for agronomic and chemical traits under Ethiopian condition and thereby promoting superior genotypes for further evaluation and cultivation.

2. MATERIALS AND METHODS

Three genotypes of spearmint (WG-SPM-Engl, WG-SPM-Fran, and SPM-EAS) maintained at Wondo Genet Agricultural Research Center and one released spearmint variety WGSM-03 used as a check were tested at Wondo Genet, Awassa, Debre Zeit and Allagae for two years in 2012 and 2013. The ecological descriptions of the testing locations are summarized under table 1. The treatments were arranged in randomized complete block design with four replications according to the procedures given by Gomez and Gomez (1984). Stolons of six months old and 10 cm in length were planted in rows of a plot 3.6 m wide and long. A spacing of 60 cm was maintained between rows. No fertilizer and chemical was applied during evaluation activity. All cultural practices and watering through flooding irrigation were done as required. Data on fresh leaf to stem ratio, fresh leaf yield (kg/ha), EO content (%) and EO yield (kg/ha) were recorded. EO content was determined on a dry weight basis from 250 g of composite leaves harvested from the three middle rows of a plot. Laboratory analyses were performed at Wondo Genet Agricultural Research Center. EO was determined by hydro-distillation (Guenther 1972). To statically analyze the differences in agronomic and chemical characteristics caused by genotypic differences, five samples were taken from each plot. Experimental data was statistically analyzed by analysis of variance (ANOVA) using SAS PROC GLM (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$.

Table 1.

Summary of site descriptions for three testing locations in Ethiopia

Testing locations	Latitude	Longitude	Soil pH	Soil type	Rainfall (mm)	Altitude (m.a.s.l)	Annual average temperature (°c)	
							Minimum	Maximum
Wondo Genet	7°19'2"N	38°38'2"E	6.4	sandy clay loam (Nitosol)	1000	1876	12.02	26.72
Aawassa	7°05'N	39°29'E	7.2	Sandy loam (Andosol)	964	1652	12.94	27.34
Allagae	7°43'N	38°36'E	8.57	Clay loam	510	1594	13.44	26.61
Debrezeit	8°44'N	38°58'E	6.9	Black heavy clay (Vertisol)	851	1891	12.22	25.72

3. RESULTS AND DISCUSSION

Mean squares from combined analysis of variance for four traits of spearmint tested over four locations of Ethiopia are summarized in tables 2. The performance of spearmint genotypes were found statistically different in all the parameters studied over the testing locations and years vary are stare The interaction effects of location and years were significant ($P < 0.05$) for leaf to stem ratio and non significant for other characters of spearmint. Location and testing years exerted a significant influence ($P < 0.05$) on leaf to stem ratio, fresh leaf yield/ha, and a highly significant influence ($P < 0.01$) on essential oil content and essential oil yield. This indicates these traits were influenced by a change in the environment. The significance of location effect was expected because Wondo genet, Allagae, Awassa and Deberezeit vary in their soil type, rainfall and temperature (Table 1). In agreement to the present study, Fehr (1991) reported that every factor that is a part of the environment of a plant has the potential to cause differential performance. Likewise, Frankel *et al.* (1994) and IRRI (1996) reported that fluctuating features of the location such as rainfall, relative humidity, temperature, etc. are some of the environmental factors that cause performance variation in plants. The influence of location on agronomic and chemical traits were also reported for coriander (Beemnet and Getinet 2010), for lemongrass (*Cymbopogon citratus* L.) (Beemnet *et al.* 2011), for Artemisia (Belay 2007) and Aflatuni (2005) for menthol mint (*Mentha arvensis* L.) and peppermint (*M. piperiata* L.), indicating the importance of knowing optimum growing locations before intending production of spearmint.

3.1. Performance Variation in spearmint genotype for agronomic and chemical characters under different growing locations and years.

Analysis of variance revealed that there existed a highly significant difference among the agronomic characters (leaf to stem ratio and leaf yield/ha) and chemical characters (essential oil content and essential oil yield) among spearmint genotypes, across testing locations and years (Table 3). The respective mean performances of agronomic and chemical characters of spearmint genotypes, location effects and influence testing years are summarized in table 4, 5 and 6.

3.2. Variation in agronomic characters of spearmint genotypes

The overall mean performance of leaf to stem ratio and leaf yield leaf yield/ha were 1.93 and 12.04 t, respectively. The respective leaf to stem ratio and leaf yield/ha of the tested genotypes ranged from 1.29 to 2.2 and 10.82 to 13.45 t and. The highest leaf to stem ratio (2.2) was demonstrated by WG-SPM-Engl and the lowest (1.29) by WG-SPM-Fran. The highest leaf yield/ha was demonstrated by WG-SPM-Fran genotype followed by WG-SPM-Engl; while the lowest value for these agronomic characters were produced by the standard check WG-SM03. Compared with the standard check (WG-SM03), WG-SPM-Engl and WG-SPM-Fran genotypes demonstrated a respective percent increase value of 15.53 and 24.31% on leaf yield/ha. Lowest fresh leaf to stem ratio was recorded for WG-SPM-Fran, though it demonstrated highest leaf yield/ha. Leaf yield/ha produced by the genotypes across different locations varied from 9.24 to 14.06 t. The highest leaf yield/ha was recorded at Hawassa (14.06 t/ha) and the lowest (9.24 t/ha) at Wondo Genet. Considering testing year, the highest leaf to stem ratio and leaf yield/plot was demonstrated during first testing year than the second. However, leaf yield/ha was found the same in both testing years. The current study is within the range of fresh herbage yield between 2.7 and 18.87 t/ha reported by Salim *et al.* (2014) for the experiment conducted under different spacing and seasonal variation in Sudan. Solomon and Beemnet (2011) reported a relatively lower leaf yield range between 3.09 and 7.48 t/ha for study conducted under different spacing and harvesting ages in Ethiopia. Similarly, lower leaf yield range from 767.8 to 815.8 kg/ha was reported by Abbaszadeh *et al.* (2009) for studies conducted in Iran for spearmint. The variation in leaf yield may be due to the variation in genotype, environment, soil, climatic factors. As the value obtained in this experiment demonstrated relatively higher values for fresh leaf yield, it is possible to cultivate spearmint in Ethiopian for the production of spearmint herbal leaf.

Table 2.

Mean square from the combined analysis of variance four spearmint genotypes tested over four locations and two years

Source of Variation	Df	Leaf to stem ratio	Leaf yield/ha	EOC (w/w fresh)	EO yield/ha
Replication	3	0.20	29540499	0.01	1267.99*
Treatments (g)	3	5.95**	44637704.9*	0.64***	13839.08***
Location (e)	3	2.64**	161010520.3***	0.03*	1120.30*
Year (y)	3	3.19*	46423498.0*	0.05*	2539.04**
g*e	9	1.55**	15157791.0ns	0.01ns	461.48 ns
g*y	1	0.51ns	20468174.3ns	0.002ns	470.14 ns
Error	105	35.94	12926847	0.01	355.797
CV (%)		29.85	29.85328	28.0501	44.003

Table 3.

Over all mean performance of four spear mint varieties tested at Wondo Genet, Allagae, Debre Zeit and Hawassa during 2012 and 2013

Treatments	Leaf to stem ratio	Leaf yield/ha (kg)	EOC (w/w fresh) (%)	EO yield/ha/year (kg)
WG-SPM-Engl	2.1862 ^a	12543.2 ^{ab}	0.39 ^b	47.875 ^b
WG-SPM-EAS	2.2047 ^a	11359.1 ^b	0.16 ^c	18.13 ^d
WG-SPM-Fran	1.2913 ^b	13449.2 ^a	0.50 ^a	67.99 ^a
WG-SM03	2.0213 ^a	10822.6 ^b	0.34 ^b	37.469 ^c
Mean	1.93	12043.54	0.35	42.87
LSD _{0.05}	0.34	1782.2	0.048	9.3502
CV (%)	35.95	29.85	28.05	24.00

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test

Table 4.

Performance of spearmint genotypes for agronomic and chemical characters tested under four different locations

Location	Leaf to stem ratio	Leaf yield/ha (kg)	EOC (w/w fresh) (%)	EO yield/ha/year (kg)
Wondo Genet	1.9 ^a	9244.1 ^c	0.35 ^{ab}	34.847 ^b
Allagae	2.113 ^a	11234.2 ^b	0.39 ^a	46.16 ^a
Debre Zeit	2.12 ^a	13634.0 ^a	0.31 ^b	42.173 ^{ab}
Hawassa	1.51 ^b	14061.8 ^a	0.34 ^{ab}	48.29 ^a
Mean	1.93	12043.54	0.35	42.87
LSD _{0.05}	0.34	1782.2	0.0484	9.3502
CV (%)	35.95	29.85	28.05	24.00

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test

Table 5.

Performance of spearmint genotypes for agronomic and chemical characters under two testing years

Testing years	Leaf to stem ratio	Leaf yield/ha (kg)	EOC (w/w fresh) (%)	EO yield/ha/year (kg)
2012	2.084 ^a	12645.8 ^a	0.37 ^a	47.32 ^a
2013	1.768 ^b	11441.3 ^a	0.33 ^b	38.413 ^b
Mean	1.93	12043.54	0.35	42.87
LSD _{0.05}	0.24	1260.2	0.0342	6.612
CV (%)	35.95	29.85	28.05	24.00

Means followed by the same letter with in the same column are statistically non significant at $P < 0.05$ according to least significant difference (LSD) test

3.3.Variation in chemical characters of spearmint genotypes

Compared among the tested genotypes, the highest essential oil content and essential oil yield was recorded for WG-SPM-Fran followed by WG-SPM-Engl; however, the lowest values were recorded for SPM-EAS and WG-SM03 (the standard check). The values for essential oil content and essential oil yield ranged from 0.16 to 0.5% and 18.13 to 67.99 kg, respectively. Compared with the standard check, WG-SPM-Engl genotype demonstrated a percent increased value of 14.7 and 27.79% on essential oil content and essential oil yield. Similarly, genotype WG-SPM-Fran demonstrated a respective percent increase value of 47.05 and 81.45% on essential oil content and essential oil yield compared with the standard check WG-SM03. The overall highest value for essential oil content (0.39%) was recorded at Allagae followed by Wondo Genet (0.35%) and Hawassa (0.34%); and the least value was recorded at Debre Zeit (0.31%). Averaged over the testing locations, the highest essential oil yield/ha (48.29 kg) was recorded at Hawassa and the lowest at Wondo Genet (34.85 kg). Compared with second testing year, a respective percent increase value of 12.12 and 23.18% on essential oil content and essential oil yield was demonstrated in first testing year.

The ranges of values obtained in the present study are in agreement within the range of different reports. A comparable range of essential oil content between 0.26 and 0.54% and essential oil yield range between 15.68 and 24.12 kg/ha was obtained was reported by Solomon and Beemnet (2011) for study conducted under different spacing and harvesting ages in Ethiopia. Likewise, a comparable essential oil content range between 1.51 and 1.52 % on dry weight basis and lower essential oil yield range values from 11.66 to 12.37 kg/ha was reported by Abbaszadeh *et al.* (2009) for studies conducted in Iran for spearmint. A relatively higher essential oil yield range between 53.8 and 83.7 kg/ha was reported by Kizil and Toncer (2006) for experimented conducted under different harvesting times in Turkey for spearmint. Salim *et al.* (2014) reported a comparable essential oil content range from 0.44 to 0.69% for spearmint tested under different growing season in Sudan. Similarly, a comparable essential oil content range from 0.32 to 0.46% and higher values of essential oil yield values between 45 and 72 kg/ha was reported for spearmint by Zheljzkov *et al.* (2010) for experiments conducted in Mississippi State University under different N application and harvesting time. The difference in essential oil content and essential oil yield may be due to climatic factors such as temperature, rainfall and light. Fahmy (1955) and Langston and Leopold (1954) mentioned that climatic factors such as temperature, day length, humidity and rainfall, affected oil content of mint plants.

CONCLUSION

Genotypes WG-SPM-Fran and WG-SPM-Engl demonstrated a respective percent increase value of 24.31 and 15.53 % on leaf yield/ha. Compared with the standard check, genotype WG-SPM-Engl demonstrated a percent increased value of 14.7 and 27.79% on essential oil content and essential oil yield. Similarly, genotype WG-SPM-Fran also demonstrated a respective percent increase value of 47.05 and 81.45% on essential oil content and essential oil yield compared with the standard check WGSM03. Hence, considering genotypes WG-SPM-Fran and WG-SPM-Engl for the production of herbal leaf and essential oil will have an economic advantage over the standard check WG-SM03.

ACKNOWLEDGEMENT

We would like to acknowledge Wondo Genet Agricultural Research Center and Aromatic and Medicinal Plants Research Project for providing all the necessary facilities and support during the entire experimentation. Our acknowledgement also to Mr. Yigermal Mola and Mr. Dantew Tsegaye of technical research assistant who collect all necessary data from all experimental fields during experimentation time and also we want to acknowledge Mr. Zerihun Jomba of laboratory technician who extracts essential oil of the plant and collect all laboratory data.

REFERENCES

- Abbaszadeh B, Alireza SV, Aliabadi HF, Hasanpour HD (2009). Studying of essential oil variations in leaves of *Mentha* species. African Journal of Plant Science 3 (10): 217-221.
- Aflatuni A (2005). The yield and essential oil content of mint (*Mentha* spp.) in Northern Ostrobothnia. PhD thesis, University of Oulu, Finland.
- Beemnet M, Getinet A (2010). Variability in Ethiopian Coriander Accessions for Agronomic and Quality Traits. African Journal of Crop Science 18(2): 43-49.
- Beemnet MK, Solomon A, Zinash TA, Hailelassie GK, Beniyam Y, Gizachew A, Bekri M, Wossen KM, Texeria DS (2011). Performance of Lemongrass (*Cymbopogon citratus* L. (DC) Stapf) for Agronomic and Chemical Traits in Different Agro-Ecologies of Ethiopia. Medicinal and Aromatic Plant Science and Biotechnology 5(2): 133-138.
- Belay B (2007). Assessment of crop growth and artemisinin content of the medicinal plant *Artemisia annua* Anamed in the Gamo highlands of SW Ethiopia. MSc. Thesis, University of Hohenheim, Stuttgart, Germany.
- Chalchat JC, Garry RP, Michet A (1997). Variation of the chemical composition of essential oil of *Mentha*

pepperiata L. during the growing time. Journal of Essential Oil Research 9: 463-465.

Edris AE, Shalaby AS, Fadel MA, Wahab A (2003). Evaluation of a chemotype of spearmint (*Mentha spicata* L.) grown in Siwa Oasis, Egypt. European Food Technology 218: 74-78.

El-Wahab, Mohamed A (2009). Evaluation of Spearmint (*Mentha spicata* L.) Productivity Grown in Different Locations under Upper Egypt Conditions. Research Journal of Agriculture and Biological Sciences 5(3): 250-254.

Fahmy IR, Saad SN, Ahmed ZF (1955). Effect of environment on yield and quality of the oil of *Mentha piperita* Grown in Egypt. chemical Abstract 51:8379-80.

Farooqi AHA, Mishra A, Naqvi AA (1983). Effects of plant age on quality of oil in Japanese mint. Indian Perfumer 27: 80-82.

Fehr WR (1991). Principles of Cultivar Development Theory and Technique. Iowa State University, USA, pp. 247-260.

Foda IM, El-Sayed MA, Hassan AA, Rasmy NM, El-Moghazy MM, (2010). Effect of Spearmint Essential Oil on Chemical Composition and Sensory Properties of White Cheese. Journal of American Science 6(5): 272-279.

Frankel OH, Brown AHD, Burdon JJ (1994). The Conservation of Plant Diversity. Cambridge University Press, UK, pp. 22-29.

Gasic O, Mitica-Dukic N, Adamovic D, Borojevic K (1989). Variability of content and composition of essential oil in different genotypes of peppermint. Biochemical Systematics and Ecology 15: 335-340.

Gomez KA, Gomez AA (1984). Statistical Procedures for Agricultural Research (2nd Ed). John Wiley and Sons, New York, pp. 1-680.

Guenther E (1972). *The Essential Oils: History-Origin in Plants Production-Analysis* (Vol I). Robert E. Kriger Publishing Co., Malabar, Florida, 427p.

Hua, C.X., Wang, G.R. and Lei, Y. 2011. Evaluation of essential oil composition and DNA diversity of mint resources from China. *African Journal of Biotechnology*, 10(74): 16740-16745.

I.R.R.I (International Rice Research Institute) (1996): Plant Adaptation and Crop Improvement. CAB International, Manila, Philippines, pp. 3-5.

Kizil S, Tonçer Ö (2006). Influence of different harvest times on the yield and oil composition of spearmint

(*Mentha spicata* L. var. *spicata*). Journal of Food, Agriculture and Environment 4 (3 and 4): 135-137.

Langston R, Leopold C (1954). Proc. Amer. Soc. Hort. Science 63: 347.

Salim EA, Gaafer M, Hassan E, Subki HEK (2014). Effect of Spacing and Seasonal Variation on Growth Parameters, Yield and Oil Content of Mint Plants. Journal of Forest Products and Industries 3(2), 71-74.

SAS (Statistical Analysis System) (2002). SAS/STAT. Guide Version 9. SAS, Institute Inc. Raleigh, North Carolina, USA

Shormin T, Khan MAH and Alamgir M (2009). Response of Different Levels of Nitrogen Fertilizer and Water Stress on the Growth and Yield of Japanese mint (*Mentha arvensis* L.). Bangladesh Journal of Scientific and Industrial Research 44(1): 137-145.

Solomon AM, Beemnet MK (2011). Row spacing and harvesting age affect agronomic characteristics and essential oil yield of Japanese mint (*Mentha arvensis* L.). Medicinal and Aromatic Plant Science and Biotechnology 5(1): 74-76.

Verma RS, Rahman L, Verma RK, Chauhan A, Yadav AK, Singh A (2010). Essential oil composition of menthol mint (*Mentha arvensis*) and peppermint (*Mentha piperita*) cultivar at different stages of plant growth from Kumaon region of western Himalaya. Open Access Journal of Medicinal and Aromatic Plants 1: 13-18.

Zheljzakov VD, Charles LC, Astatkie T, Wayne ME (2010). Productivity, Oil Content, and Composition of Two Spearmint Species in Mississippi. Agronomy Journal 102(1): 129-133.

Znini M, Boukhal M, Majidi L, Kharchouf S, Aouniti A, Bouyanzer A, Hammouti B, Costa J, Al-Deyab SS (2011). Chemical Composition and Inhibitory Effect of *Mentha Spicata* Essential Oil on the Corrosion of Steel in Molar Hydrochloric Acid. International Journal of Electrochemical Science 6: 691-704.

Silva E, Pedro Mde A, Sogayar AC, Mohovic T, Silva CL, Janiszewski M, Cal RG, de Souza EF, Abe TP, de Andrade J, de Matos JD, Rezende E, Assunção M, Avezum A, Rocha PC, de Matos GF, Bento AM, Corrêa AD, Vieira PC, Knobel E; Brazilian Sepsis Epidemiological Study. Brazilian Sepsis Epidemiological Study (BASES study). Crit Care. 2004;8(4):R251-60. Comment in Linde-Zwirble WT, Angus DC. Severe sepsis epidemiology: sampling, selection, and society. Crit Care. 2004;8(4):222-6.

Alberti C, Brun-Buisson C, Chevret S, Antonelli M, Goodman SV, Martin C, Moreno R, Ochagavia AR, Palazzo M, Werdan K, Le Gall JR; European Sepsis Study Group. Systemic inflammatory response and progression

to severe sepsis in critically ill infected patient. *Am J Respir Crit Care Med*. 2005;171(5):461-8.

Eliézer Silva, Luiz Dalfior Junior, Haggéas da Silveira Fernandes, et al. Prevalence and outcomes of infections in Brazilian ICUs: a subanalysis of EPIC II study. *Rev. bras. ter. intensiva* vol.24 no.2 São Paulo Apr./June 2012

Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System. *Crit Care Med* 1999; 27: 887-92.

Ponce de Leon-Rosales SP, Molinar-Ramos F, Dominguez-Cherit G, Rangel-Frausto M, Vazquez-Ramos VG. Prevalence of infections in intensive care units in Mexico: A multicentre study. *Crit Care Med* 2000; 28: 1316-21.

Avecillas J, Mazzone P, Arroliga A. A rational approach to the evaluation and treatment of the infected patient in the intensive care unit. *Clin Chest Med* 2003; 24: 645-69.