

PBRG
Plant Biotechnology Research Group

Institute for Biotechnology and Bioengineering
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Plant Biotechnology Research Group

Objectives

The Plant Biotechnology Research Group (PBRG) is a research unit on plant sciences also involved in advanced education on biotechnology. The main research goals, on a pluriannual basis, are the *in vivo* and *in vitro* secondary metabolites production, aiming the assessment of the National endemic aromatic/ medicinal flora potentialities, the molecular characterization of aromatic species, focusing on the terpene synthase genes, the study of plant secretory structures and the prospecting of bioactive components. Briefly, the following topics have been under study during 2011:

Research Topics

1. a) Chemosystematic survey of Portuguese propolis volatiles (col. CIMO-IPB/FCUP), b) Volatiles characterization and acaricide detection in Propolis from beehives maintained at Algarve, c) Characterization of the volatiles from *leiva*, a warm organic matter-rich soil used for growing pineapples (*Ananas comosus*) in São Miguel (Azores, Portugal) (Col. CBA), and d) Characterization of the chemical composition and biological activity of the volatiles from *Ormenis mixta* and *Paronychia argentea* (Transnational Cooperation Portugal-Morocco),
2. a) Establishment of *in vitro* co-culture model systems of micropropagated *Pinus* spp./



Bursaphelenchus xylophilus (Pine Wilt Nematode) and *Solanum tuberosum* hairy roots/ *Meloidogyne chitwoodi* (Potato-Cyst Nematodes) for characterization of the nematodes invasion mechanisms, and b) establishment of micropropagated *Thymus caespititius* to assist on the molecular elucidation of essential oil polymorphism in this species,

3. a) Searching for TPS genes in *Thymus caespititius* based on conserved regions available for other Lamiaceae, starting with primer design and RACE-PCR, leading to cloning the full-sequence gene, and b) construction of a cDNA library of *T. caespititius* in order to establish its relationship with the different chemotypes in this species, and b) Study of heterologous expression of previously identified TPS genes: TPS1 and TPS2,
4. Study of the morphology, anatomy, histochemistry and ultrastructure of the secretory structures of medicinal and/or aromatic plants,
5. Evaluation of the biological activities of aqueous plant extracts, infusions and decoctions, and isolated phenolic constituents, that could explain some of the traditional uses of the medicinal species (col. CQB-FCUL, CBA-FCUL),
6. Nutritional quality evaluation of fresh and fresh-cut fruit and their changes throughout the storage period,

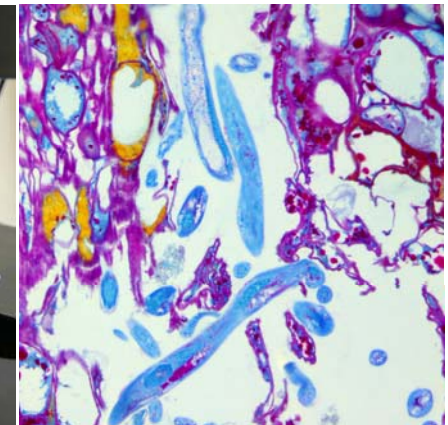


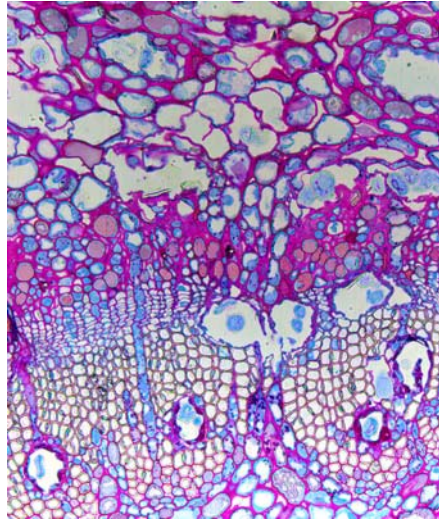
8. Identification of the catabolic products of β -myrcene used as sole carbon source by *Pseudomonas* sp. M1 and their correlation with bacterial gene expression.

Major Achievements

- Pursuing our studies in the *Plectranthus* genus, it was demonstrated that *P. barbatus* herbal tea and its main constituent (rosmarinic acid), administered intragastrically or intraperitoneally to rats, can cross the intestinal barrier, as well as the blood brain barrier and be detected in the brain, where it inhibits the enzyme acetylcholinesterase. It was also showed that the metabolization and bioavailability of the tea components is different from the administration of pure compounds. In addition, protein intrinsic fluorescence analysis proved that *P. barbatus* extract components and its metabolites (rosmarinic acid, luteolin and apigenin), found in the rat plasma, are able to bind to the human transport proteins albumin and lysozyme, allowing them to be carried in the bloodstream to different organs. *P. barbatus* tea constituents also inhibit lysozyme activity. The inhibition of lysozyme along with the radical scavenger capability, previously shown, suggests that *P. barbatus* water extract is a very promising anti-inflammatory raw material (col. CQB, FCUL).

7. a) Assessment of essential oils, and their fractions, for their potential nematocidal properties, namely in the control of the pine wood nematode, *Bursaphelenchus xylophilus*, b) Chemical characterization of Portuguese *Pinus pinaster* and *P. pinea* volatiles from both healthy and *in lab* mechanically wounded uninoculated and inoculated plants with nematodes, and c) evaluation of the histological and cellular changes induced in pine tissues (*P. pinaster* and *P. pinea*) by a virulent strain of *B. xylophilus* (collab. NemaLab – ICAM, Univ. Évora),





- Different solvent-extracts from several aromatic species were investigated for their antioxidant and anti-inflammatory activities. *S. officinalis* extracts were the best in scavenging DPPH radicals and superoxide anion radicals and it was verified that the time of hydrodistillation influenced the antioxidant activity. *O. vulgare* and *T. mastichina* extracts were also effective as DPPH and superoxide scavengers, respectively. Deodorized chloroformic extracts from *T. mastichina*, *T. camphoratus*, *T. carnosus*, *O. vulgare*, *F. vulgare* and *S. officinalis* exhibited high lipoxygenase activity inhibition.



- The essential oils from several Portuguese *Thymus* species, showed a quite broad variation in terms anti-acetylcholinesterase activity. Moreover, *T. zygis* subsp. *zygis* essential oil showed the strongest acetylcholinesterase inhibition capacity with an IC50 = 1.1µg/ml.
- *T. caespititius* essential oil from Planalto Central (Pico, Azores) and its main component, carvacrol significantly ($p < 0.05$) inhibited the intracellular growth of *H. pylori*, and showed no cytotoxicity to the gastric cell line used.
- The evaluation of antioxidant activity of hydro-alcoholic extracts of propolis, from different zones of Algarve, showed a coincidence with the highest amounts of phenols present in the extracts.
- A high genetic variability was observed among individuals of *Pittosporum undulatum* that can explain the invasive ability of this species in the Azores archipelago. The detailed characterization of this alien weed species may assist finding its potential commercial uses as well as management strategies to help its eradication and/or control.
- In the assays for testing the nematocidal properties of essential oils, it was noticed that acetone seems to be an appropriate alternative solvent vehicle for this kind of experiments. Moreover, the essential oil of *Ruta graveolens* showed a notorious activity (col. NemaLab – ICAM, Univ. Évora).
- The evaluation of quality changes throughout the storage period showed that citric acid



treatment was better for preserving firmness, and 2% ascorbic acid dip was better for retaining the nutritional properties of fresh-cut kiwifruit.

- Identification and characterization of 2 putative TPS genes in *T. caespititius*, *Tctps1*, *Tctps2*, putatively encoding for sabinene synthase and γ -terpinene synthase, respectively. Gene structure, intron and exon placement was confirmed by comparing cDNA and gDNA sequence information. The partial characterization of *Tctps3*, a putative gene coding for 1,8-cineole synthase, was also achieved.
- Study of *Tctps1* and *Tctps2* expression on *T. caespititius* micropropagated cultures belonging to different chemotypes.
- *Tctps1* and *Tctps2* were also identified and characterized on *T. caespititius*, of different chemotypes, established in the field.
- Preliminary study of heterologous expression of *Tctps2* was performed (col. CEB/IBB, U. Minho).

Selected Publications

- Dandlen S.A., M.G. Miguel, J.M. Duarte, M.L. Faleiro, M.J. Sousa, A.S. Lima, A.C. Figueiredo, L.G. Pedro, J.G. Barroso. Acetylcholinesterase inhibition activity of Portuguese *Thymus* species essential oils. *Journal of Essential Oil Bearing Plants*, 14: 140-150.
- Dandlen S.A., A.S. Lima, M.D. Mendes, M.G.

Miguel, M.L. Faleiro, M.J. Sousa, L.G. Pedro, J.G. Barroso, A.C. Figueiredo. Antimicrobial activity, cytotoxicity and intracellular growth inhibition of Portuguese *Thymus* essential oils. *Brazilian Journal of Pharmacognosy*, 21: 1012-1024.

Falé P.L.V., P.J.A. Madeira, M.H. Florêncio, L. Ascensão, M.L.M. Serralheiro. Function of *Plectranthus barbatus* herbal tea as neuronal acetylcholinesterase inhibitor. *Food Function*, 2: 130-136.

Mendes M.D., A.S. Lima, H. Trindade, A.I.D. Correia, J.G. Barroso, L.G. Pedro, A.C. Figueiredo. ISSR molecular characterization and leaf volatiles analysis of *Pittosporum undulatum* Vent. naturalized in the Azores archipelago (Portugal). *Industrial Crops and Products*, 33: 710-719.

Miguel G., C. Cruz, M.L. Faleiro, M.T.F. Simões, A.C. Figueiredo, J.G. Barroso, L.G. Pedro. *Salvia officinalis* L. essential oils: effect of hydrodistillation time on the chemical composition, antioxidant and antimicrobial activities. *Natural Product Research*, 25: 526-541.



Publications

Articles in International Peer-Reviewed Journals

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Albano S., M.G. Miguel, Biological activities of extracts of plants grown in Portugal. *Industrial Crops and Products*, 33: 338-343.

Belhatab R., M. Boudjouref, J.G. Barroso, L.G. Pedro, A.C. Figueirido, Essential oil composition from *Artemisia campestris* grown in Algeria. *Advances in Environmental Biology*, 5: 429–432.

Dandlen S.A., M.G. Miguel, J.M. Duarte, M.L. Faleiro, M.J. Sousa, A.S. Lima, A.C. Figueiredo, L.G. Pedro, J.G. Barroso, Acetylcholinesterase inhibition activity of Portuguese *Thymus* species essential oils. *Journal of Essential Oil Bearing Plants*, 14: 140-150.

Dandlen S.A., A.S. Lima, M.D. Mendes, M.G. Miguel, M.L. Faleiro, M.J. Sousa, L.G. Pedro, J.G. Barroso, A.C. Figueiredo, Antimicrobial

activity, cytotoxicity and intracellular growth inhibition of Portuguese *Thymus* essential oils. *Brazilian Journal of Pharmacognosy*, 21: 1012-1024.

Falé P.L.V., L. Ascensão, M.L.M. Serralheiro, P.I. Haris, Interaction between *Plectranthus barbatus* herbal tea components and human serum albumin and lysozyme: binding and activity studies. *Spectroscopy*, 26:79-92.

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Figueiredo A.C. and L.G. Pedro, Book Review: Protocols for *In Vitro* Cultures and Secondary Metabolite Analysis of Aromatic and Medicinal Plants. S. Mohan Jain and Praveen K. Saxena (Eds), Humana Press, ISBN 978-1-60327-286-5. *Flavour Fragrance Journal*, 26: 70.

Gago C., A.R. Sousa, M. Juliao, G. Miguel, D. Antunes, T. Panagopoulos, Sustainable use of energy in the storage of halophytes used for food. *International Journal of Energy and Environment*, 5: 592-599.

Mendes M.D., A.S. Lima, H. Trindade, A.I.D. Correia, J.G. Barroso, L.G. Pedro, A.C. Figueiredo, ISSR molecular characterization and leaf volatiles analysis of *Pittosporum undulatum* Vent. naturalized in the Azores archipelago (Portugal). *Industrial Crops and Products*, 33: 710-719.

Miguel G., C. Cruz, M.L. Faleiro, M.T.F. Simões, A.C. Figueiredo, J.G. Barroso, L.G. Pedro, *Salvia officinalis* L. essential oils: effect of hydrodistillation time on the chemical composition, antioxidant and antimicrobial activities. *Natural Product Research*, 25: 526–541.

Miguel M.G., Anthocyanins: Antioxidant and/or anti-inflammatory activities. *Journal of Applied Pharmaceutical Science*, 1: 7-15.



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Palavra A.M.F., J.P. Coelho, J.G. Barroso, A.P. Rauter, J.M.N.A. Fareleira, A. Mainar, J.S. Urieta, B.P. Nobre, L. Gouveia, R.L. Mendes, J.M.S. Cabral, J.M. Novais, Supercritical carbon dioxide extraction of bioactive compounds from microalgae and volatile oils from aromatic plants. *Journal of Supercritical Fluids*, 60: 21-27.

Pestana M, I. Domingos, F. Gama, S. Dandlen, M.G. Miguel, J.C. Pinto, A. de Varennes, P.J. Correia, Strawberry recovers from iron chlorosis after foliar application of a grass-clipping extract. *Journal of Plant Nutrition and Soil Science*, 174: 473-479.

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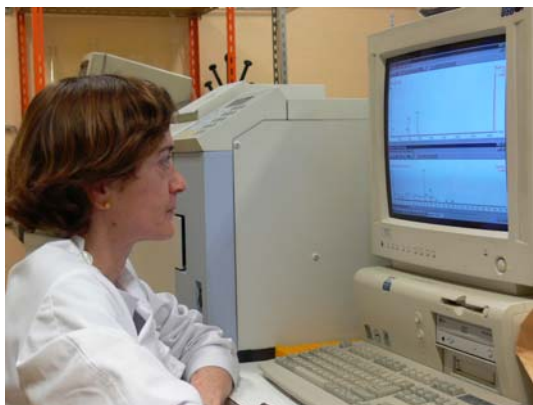
commercial essential oils and their major compounds. *Molecules*, 16: 7672-7690.

Smail A., B. Lyoussi, M.G. Miguel, Antioxidant activity of some Moroccan hydrosols. *Journal of Medicinal Plants Research*, 5: 6688-6696.

Book chapters

Barata A.M., F. Rocha, V. Lopes, E. Bettencourt, A.C. Figueiredo, Medicinal and Aromatic Plants – Portugal, In: *Medicinal and Aromatic Plants of The World*, [Eds. M. Ozturk, G.-F. B. Ameenah], Encyclopedia of Life Support Systems (EOLSS), Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, UK, [http://www.eolss.net].

Miguel M.G., Virgin olive oil: composition and biological properties. In Fixed oils and fats of pharmaceutical importance. *Recent Progress in Medicinal Plants*. Vol. 33, chapter 10, Studium Press LLC, USA. Pp. 287-322.



Other Communications

International Conferences

Aazza S., B. Lyoussi, M.G. Miguel, A.C. Figueiredo, L.G. Pedro, J.G. Barroso (2011) Antioxidant and acetylcholinesterase inhibitory activity from *Citrus aurantium* L., *Citrus limon* (L.) Burm. f., *Cupressus sempervirens* L., *Eucalyptus globulus* Labill., *Foeniculum vulgare* Mill. and *Thymus vulgaris* L. Moroccan essential oils. 42th International Symposium on Essential Oils, Antalya, Turkey, p. 72.

Anas E., A. El Amrani, J.J. Eddine, J.G. Barroso, L.G. Pedro, A.C. Figueiredo (2011) Yield and chemical composition of the essential oil from *Ormenis mixta* grown in Morocco. 4ème Symposium International Plantes Aromatiques e Médicinales (SIPAM), Mohammedia, Morocco, p. 160.

Antunes M.D.C., D.A.C. Rodrigues, A.M. Cavaco, M.G. Miguel (2011) Evaluation of Quality Changes during Shelf-life in Minimally Processed Kiwifruit. *Acta Horticulturae*, 913: 553-557.

Bahcevandziev K., A.S. Lima, H. Trindade (2011) -Estimation of nuclear DNA content in various chemotypes of *Thymus caespititius* Brot. (Lamiaceae) by flow cytometry, 1st Annual Conference, Plant Engine, Cost Meeting, Action FA1006, Murcia, Spain, p: 28

Barbosa P., J.M.S. Faria, M.D. Mendes, L.S. Dias, J.G. Barroso, L.G. Pedro, A.C. Figueiredo, M. Mota (2011) Search for pinewood nematode control using essential oils isolated from Portuguese aromatic flora. XLIII ONTA Annual Meeting, Coimbra, Portugal, p. 160.

Belhattab R., L. Amor, J.G. Barroso, L.G. Pedro, A.C. Figueiredo (2011) Essential oil from *Artemisia herba-alba* Asso grown wild in Algeria: variability assessment and comparison



with an updated literature survey, Fourth International Chemical Conference, Riyadh, Saudi-Arabia, p. 146.

Falcão S.I., A.C. Figueiredo, M. Vilas-Boas (2011) Volatile composition of Northeast Portuguese propolis. 1º Congresso Ibérico de Apicultura, Castelo Branco, Portugal, pp. 89-90.

Falé P.L.V., M.A. Filipe, L. Ascensão, M.L.M. Serralheiro, L. Mira (2011). Activity of *Plectranthus barbatus* extract against inflammatory response in human neutrophils. International PSE Symposium - Phytochemicals in Nutrition and Health, Bari, Itália.

Faria J.M.S., A.C. Figueiredo (2011) Comparative evaluation of the volatiles from micropropagated and two-years-old field grown *Pinus pinaster* and *Pinus pinea* plants. 7th International Symposium on In Vitro Culture and Horticultural Breeding, Ghent, Belgium, p. 43-44.

Faria J.M.S., P. Barbosa, M. Mota, J. Sanches, A.C. Figueiredo (2011) Evaluation of the nematicidal activity against the pinewood nematode, *Bursaphelenchus xylophilus*, of essential oil-bearing plants from Portuguese flora. XLIII ONTA Annual Meeting, Coimbra, Portugal, p. 162.

Feijão M.D., G. Teixeira, A.I. Correia, L.G. Pedro, J.G. Barroso, A.C. Figueiredo (2011) Essential oils from *Lavandula luisieri* (Rozeira) Rivas-Martinez grown at a montado ecosystem. 42th International Symposium on Essential Oils, Antalya, Turkey, p. 178.

Feijão M.D., G. Teixeira, T. Vasconcelos, L. Rodrigues, A.I. Correia, J. Sanches, L.G. Pedro, J.G. Barroso, A.C. Figueiredo (2011) Essential oil variability and trichomes morphology from *Lavandula pedunculata* (Mill.) Cav. grown at Mata Experimental do Escaroupim (Portugal). 59th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA 2011), Antalya, Turkey, p. 1297.

Figueiredo A.C., L.G. Pedro, J.G. Barroso (2011) Hairy root cultures and the in vitro production of volatiles: an update. 42th International Symposium on Essential Oils, Antalya, Turkey, p. 32.

Figueiredo A.C., L.G. Pedro, J.G. Barroso (2011) Volatile phytochemicals production by hairy root cultures. *Plant Engine – COST Action FA 1006*, Murcia, Spain, p. 70.

Francisco A., L. Ascensão (2011). Osmophores in the labellum of three *Ophrys* species: structure and role in pollination of sexually deceptive orchids. 18th International Botanical Congress, Melbourne, Austrália.

Francisco A., L. Ascensão (2011). Structure and function of the osmophores in three species of *Ophrys*, a genus of sexually deceptive pollinated orchids. 2nd Joint Congress of Portuguese and Spanish Microscopy Societies, Aveiro, Portugal.

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Lopes V.R., A.M. Barata, F. Rocha, L.G. Pedro, J.G. Barroso, A.C. Figueiredo (2011) Morphological and chemical variability assessment from Portuguese *Mentha pulegium* L. (pennyroyal) accessions. Proceedings of the VIII International Ethnobotany Symposium, Lisboa, Portugal, p. 573-583.

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Marin M., L. Ascensão, S. Duletic-Lausevic, P.A. Marin (2011) Light and electron microscopy in investigation of glandular trichomes of *Satureja montana* L. 10th Multinational Congress on Microscopy (MCM 2011), Urbino, Itália.

Mendes M.D., A.C. Figueiredo, M.M. Oliveira, H. Trindade (2011) Expression of a putative monoterpene synthase along *Thymus caespititius* micropropagation. 7th International Symposium on In Vitro Culture and Horticultural Breeding, Ghent, Belgium, p. 63.

Mota L., A.C. Figueiredo, L. Pedro, J. Barroso, M.G. Miguel, L. Faleiro, L. Ascensão (2011) Chemical composition, antioxidant and antimicrobial activities of volatiles from *Plectranthus barbatus*, *P. neochilus* and *P. ornatus* (Lamiaceae). 42th International Symposium on Essential Oils, Antalya, Turkey, p. 129.

Panagopoulos T., M.D.C. Antunes (2011) Spatial distribution of qualitative characteristics and production factors of kiwifruit at "Beira Litoral", Portugal. *Acta Horticulturae*, 913: 609-614.

Panagopoulos T., C. Gago, A.R. Sousa, M. Juliao, G. Miguel, M.D.C. Antunes (2011) Exploiting halophytes from Ria Formosa (Portugal) for fresh salads. 2nd Conference of COST Action FA0901 'Ecology of halophytes and saline habitats', Torun, Poland.

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Smail A., L. Badiãa, M.G. Miguel, A.C. Figueiredo, L.G. Pedro, J.G. Barroso (2011) Antioxidant and acetylcholinesterase inhibitory activity from *Citrus aurantium* L., *Citrus limon* (L.)



Burm. f., Eucalyptus globulus Labill., *Foeniculum vulgare* Mill. and *Thymus vulgaris*. 42nd International Symposium on Essential Oils, Antalya, Turquia

National Conferences

Bahcevandziev K., R. Guilherme, F. Melo, P. Mota, H. Trindade (2011) Estudo preliminar de quimiotipos de *Thymus caespititius* com recurso à técnica da citometria de fluxo, 3º Colóquio Nacional de Horticultura Biológica (Braga), pp: 42

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Dias, M.I., I. Ferreira, H. Trindade, M.J. Sousa (2011) Caracterização química e molecular de amostras de *Coriandrum sativum* L. obtidas in vivo e in vitro. I Jornadas de Jovens Investigadores da Escola Superior Agrária de Bragança, Nov.11-12.

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Rodrigues A.M., P.L.V. Falé, L. Ascensão, A.R. Lino, M.L.M. Serralheiro (2011). Actividades biológicas de três espécies da flora aromática Portuguesa. 1º Encontro Ciência do Centro de Química e Bioquímica, Lisboa, Portugal. (winner of the best poster award).

Pedro L.G., A.C. Figueiredo, J.G. Barroso (2011) Métodos de obtenção dos óleos essenciais, V Curso de Plantas Aromáticas e Medicinais em Terras do Pulo do Lobo, Mértola.

PhD. Thesis

Dayana Maria Teodoro Francino, "Anatomia foliar de *Chamaecrista* Moench. (Leguminosae-Caesalpinioideae) como subsídio à taxonomia e à filogenia". Supervisors: Lia Ascensão, (CBV, FCUL, UL), Renata Meira and Aristéa Alves Azevedo (DB, Federal Univ of Viçosa, MG, Brasil).

M.Sc. Thesis

Ana Margarida Rodrigues, "Actividades Biológicas e Estruturas Secretoras em Três Espécies de Asteraceae da Flora Aromática Portuguesa". Mestrado em Biologia Celular e Biotecnologia (FCUL). Supervisors: Luísa Serralheiro (CQB, FCUL), Lia Ascensão (CBV/IBB, FCUL)

Inês Correia dos Santos Carvalho de Sena, "Avaliação da diversidade genética de populações de *Tuberaria major* usando marcadores de SSR". Mestrado Integrado em Engenharia Biológica (Universidade do Algarve). Supervisors: Anabela Romano (CGB/IBB, UAlg) e Helena Trindade (CBV/IBB, FCUL).

Inês Margarida Pereira Ramos, "Percepção da

qualidade dos produtos hortícolas produzidos na Póvoa de Varzim", Mestrado em Hortofruticultura (Universidade do Algarve). Supervisors: Maria Dulce Carlos Antunes (CBV/IBB, UALG) e Maria Raquel Lucas (UE).

Maria Inês Moreira Figueiredo Dias, "Caracterização química e molecular de amostras de *Coriandrum sativum* L. obtidas in vivo e in vitro", Mestrado em Biotecnologia (Escola Superior Agrária de Bragança). Supervisors: Maria João Sousa e Isabel Cristina Ferreira (ESA) e Helena Trindade (CBV/IBB, FCUL).

Marta Taveira Santos Castro Silva, "Avaliação da capacidade de extractos voláteis de plantas aromáticas para inibir a formação de biofilmes bacterianos", Mestrado em Biologia Celular e Biotecnologia Vegetal (FCUL). Supervisors: Carla da Conceição Caramujo Rocha de Carvalho (BERG/IBB, IST) e Ana Cristina Figueiredo (CBV/IBB, FCUL)

Natacha Sofia Greno de Moura, "Usos tradicionais e avaliação da composição química dos óleos essenciais de plantas aromáticas e medicinais utilizadas em Lisboa e Bragança", Mestrado em Biologia Celular e Biotecnologia Vegetal (FCUL). Supervisors: Ana Carvalho (Instituto Politécnico de Bragança), Ana Cristina Figueiredo (CBV/IBB, FCUL)

