Utilisation and Management of Medicinal Plants
Dedicated to

Prof. C. K. Atal
Former Director, Indian Institute of Integrative Medicine, Jammu
(Erstwhile, Regional Research Laboratory, Jammu)
Foreword

With the growing demand and popularity of herbal drugs and the wide use of plant derived phyto-molecules in modern medicine, in recent times, there has been great demand for medicinal plants in the drug industry.

The gap between demand and supply are widening in the herbal drug industry. Until recently, the demands for raw materials were mostly met from wild naturally growing plants. Owing to various reasons, particularly because of habitat destruction and shrinking of forest area, it is no longer possible to meet the requirement of medicinal plants from wild or natural sources. This book—Utilisation and Management of Medicinal Plants edited by Dr. Anil K. Verma and Dr. Sushma Koul with Dr. V.K. Gupta as Editor-in-chief is a valuable and timely attempt to bring together expert opinions and ideas for the Utilisation and Management of Medicinal Plants in a single volume.

There are twenty one chapters in this book. The editors have invited eminent scientists from all over the world to contribute chapters for the book. The contributed chapters cover a wide range of subjects related to the management and utilisation of medicinal plants such as biotechnology, phytochemistry, plant-physiology, fungal metabolites, bioactivity of secondary metabolites, antibacterial and anti-fungal activity of secondary metabolites, bio-prospecting etc. Each chapter in the book is contributed by eminent scientists who have been working in the relevant areas for a considerable period.
This book, I am glad to see, is dedicated to Dr. C.K. Atal, a towering figure in the study of medicinal plants and their bioactive secondary metabolites. As Director of Regional Research Laboratory, Jammu, which is now known as Indian Institute of Integrative Medicine, Dr. Atal inspired a generation of young scientists to devote their life in the study of medicinal and aromatic plants. Under his leadership and guidance, several scientific discoveries in natural sciences, especially discovery of a large number of bioactive molecules, novel mechanisms of drug action etc were carried out. He instilled confidence and inspiration in his colleagues, co-workers and students. Being a visionary with a brilliant, intuitive and analytical mind, Dr. Atal, encouraged his co-workers to tread untravelled paths which yielded great dividends in terms of research publications, discovery of novel bioactivities, molecules, new drugs and perfumery products, patents and recognition. Prof. Atal has been known to me from 1969 onwards. I had the privilege of working in his team from 1969 to 1989 at IIIM. All these years, I had very close association with Prof. (Dr.) C.K. Atal. It is with his blessings and good wishes that I have grown up in the scientific ladder. Hence I have immense pleasure in writing this foreword to a publication dedicated to Dr. C.K. Atal.

I complement the editors as well as the authors for their valuable contribution. This book will be of immense value and use to students, researchers and professionals engaged in the study and utilisation of medicinal and aromatic plants.

P. Pushpangadan
Preface

Nature has provided mankind with products for good health since the beginning of time. For thousands of years medicine and natural products have been closely linked through the use of traditional medicines and natural poisons (Newman et al., 2000). Plants hold a prominent position in the available sources of natural bioactive molecules. Clinical, pharmacological, and chemical studies of traditional medicines, derived predominantly from plants, were the basis of most early medicines such as aspirin, digitoxin, morphine, quinine, emetine, and pilocarpine. Approximately 25 per cent of the drugs prescribed worldwide come from plants, whereas 11 per cent of the 252 drugs considered as basic and essential by the World Health Organization (WHO) derive their origin exclusively from plants. Prescription drugs containing phytochemicals were valued at more than US$ 30 billion in 2002 in the US alone.

Eighty percent of the people in developing countries rely on traditional medicines for primary health care. Development of phyto-therapeutic during last few decades has evolved into a science itself and is undergoing further change. The traditional, complementary and the alternative medicine is attracting more and more attention within the context of health care provision and health sector reforms. Many of the Botanicals as ingredients have histories of prior human. These components are already being used in food as dietary ingredients, in traditional system of medicine for different ailments or as food and medicine both. Obliviously the food substances are considered to be safe and nourishing, for those which are having medicinal properties and are described for treatment of specific alignments, proper procedure to eliminate adulteration, contamination and toxic side effects are needed. Worldwide need of alternative medicine and growth of natural product inserts the traditional systems of medicine into markets. The traditional medicines are slowly being integrated into modern medicine in the form of dietary and nutritional supplements and the scientific community is challenged to address the issues of utility, safety, quality efficacy and standardization.
During the last few decades, public interest in natural therapies has increased tremendously around the world and natural bioresource as therapeutic has evolved into a science and is undergoing faster changes. The challenge today is transforming the long history of experience-based medicine in evidence based medicine. Indeed, the lack of readily accessible information on how much of what medicinal plant biodiversity occurs where has become a limiting factor in conservation planning and converting our biowealth into economic wealth. Over harvesting from forests, deforestation and other anthropogenic factors have already placed several species at risk.

Since new diseases as well as drug-resistant strains of known pathogens continue to emerge, the search for novel compounds in combating many diseases and conditions is continuing globally. The scientific integration of herbal medicinal into modern medical practices must take into account the interrelated issues of quantitative and qualitative assessment of bioresource, mass production and its appropriate use. The current worldwide trend towards the utilisation of natural remedied has created an enormous need for information about the properties and uses of natural resources.

In the present book “Utilisation and Management of Medicinal Plants” an attempt has been made to encapsulate scientific information pertaining to modern-day drug research and this effort is likely to serve as a catalyst for the development of innovative methodologies and approaches for further studies, paving the way for discovery of novel drug for various human ailments.

The topics have been contributed by the experts in the field with exhaustive, relevant and up-to-date information. It is hoped the book be a useful resource of knowledge for ethnobotanists, pharmacologists, biochemists, physiologists, agronomists, medicinal researchers, pharmaceutical scientists and people of allied disciplines engaged in research new drugs of plant origin. We are greatly indebted to the scientists and researchers who have contributed for this volume.

V. K. Gupta
Anil K. Verma
Sushma Koul
Contents

Foreword vii
Preface ix

1. Biotechnological Interventions for Enhancing the Availability of High Value Medicinal Plants
   Nishritha Bopana and Sanjay Saxena 1

2. Environmental Regulation of Secondary Metabolite Accumulation in Aromatic Crops: A Review
   K. Ramesh and Virendra Singh 32

3. Search for Useful Substances in Papyrus Cyperus papyrus L.
   Nariaki Wakiuchi, Hajime Tamaki, Takashi Nishino and Yukihiro Sugimoto 48

4. Secondary Plant Products and their Biopotentials
   S.C. Jain, R. Singhl and R. Jain 55

5. Bioactive Compounds from Fungi: A Review
   Nilanjana Das and Lazar Mathew 107

6. Elevated Carbon Dioxide Levels Enhance Rosmarinic Acid Production in Spearmint Plantlets
   Brent Tisserat, Mark Berhow and Steven F. Vaughn 124

7. Approaches for Enhancing Yield of Artemisinin: A Novel Antimalarial Compound, in Artemisia annua L. Plants
   M.Z. Abdin, Mauji Ram, Usha Kiran and M.A. Khan 136
8. *Pieris brassicae* as Laboratory of Synthesis of New Compounds with Biological Potential: Interaction with *Brassica oleracea var. costata* and *Brassica rapa var. rapa*. 157
   *David M. Pereira, Patrícia Valentão, Federico Ferreres, Rosa Seabra and Paula B. Andrade*

   *P.O. Osadebe, I.C. Uzochukwu and E.O. Omege*

10. Comparative Assessment of Growth Productivity and Chemical Profiling of Wild and Cultivated Populations of *Withania somnifera* (L.) Dunal. 183
    *Arun Kumar, M.K. Kaul, Punit Kumar Khanna, Sushma Koul and K.A. Suri*

11. Bioprospecting: A Move from Land to the Seas 202
    *Kushal Qanungo*

12. The Genus Mentha: A Versatile Plant 217
    *Amrita Chakraborty, Krittika Sasmal and Sharmila Chattopadhyay*

13. Medicinal and Aromatic Potentialities of *Ocimums* and their Efficacy in Traditional Systems of Medicine 228
    *M.K. Khosla and V.K. Gupta*

14. Herbs that can Compact Noise Stress: A Mini Review 244
    *R. Sheeladevi*

15. Bioactive Compounds from *Annona* Species 278
    *Beena Joy*

16. Saffron: A High Value Crop 294
    *Sushma Koul, Esha Abrol, Bilal A. Mir and A.K. Koul*

17. Antibacterial Activity of *Acacia daviesii* (M. Bartolome) sp. nov.: A New and Rare Species Identified in North-Eastern Victoria, Australia 306
    *Hilde Lie Kjaerstad, Allison McGill and Enzo A. Palombo*

18. A Review on Some Potential Anthelmintic Herbal Drugs 319
    *Ravindra G. Mali*

19. Variations in Tannin and Oxalic Acid Content in *Terminalia arjuna* (Arjuna) Bark 340
    *A.K. Pandey and D.C. Kori*

20. Natural Sweetening Agents: A Review 348
    *Annie Shirwaikar, Arun Shirwaikar, Richard Lobo and Kirti S. Prabhu*

    *Diby Paul and Y.R. Sarma*

Index 377
Medicinal plants have extensive past and present use in treatment of diverse diseases and serve as compounds of interest both in their natural form and as templates for synthetic modifications. According to an estimate of the World Health Organization (WHO), approximately 80 per cent of the people in developing countries rely chiefly on traditional medicines for primary health care needs; a major portion of these involves the use of medicinal plants.

The present book “Utilisation and Management of Medicinal Plants” encompass 21 research and review articles contributed by eminent researchers/scientists from various parts of the globe and covers the importance of scientific research on medicinal plants in the areas of botany, chemistry, biotechnology, toxicology and pharmacology. Following are some of the lead articles included in this book:

- Biotechnological Interventions for Enhancing the Availability of High Value Medicinal Plants
- Environmental Regulation of Secondary Metabolite Accumulation in Aromatic Crops
- Search for Useful Substances in Papyrus Cyperus papyrus
- Secondary Plant Products and their Biopotentials
- Bioactive Compounds from Fungi
- Approaches for Enhancing Yield of Artemisinin
- Pieris brassicae as Laboratory of Synthesis
- Bioprospecting: A Move from Land to the Seas
- Medicinal and Aromatic Potentialities of Ocimums
- Medicinal Properties of Mistletoe
- Saffron: A High Value Crop
- Some Potential Anthelminitic Herbal Drugs
- Variations in Tannin and Oxalic acid content in Terminalia arjuna
- Natural Sweetening Agents

It is hoped that this book with wide spectrum of themes shall generate further interest and database for the benefit of academicians, researchers/scientists working on medicinal plants who shall find it very useful and indispensable in their relevant research pursuits.
Dr. Vijay Kumar Gupta (born 1953-) obtained his Masters (1975) and Doctorate (1979) from University of Jammu, Jammu-India and is serving as Deputy Director and Head, Animal House, Indian Institute of Integrative Medicine (CSIR), Jammu, India. His research capabilities are substantiated by his excellent work on histopathology, ecology and reproductive biology of fishes, turtles, birds and mammals, which has already got recognition in India and abroad.

Dr. Gupta has to his credit more than 75 scientific publications and review articles which have appeared in internationally recognized Indian and foreign journals. Founder fellow, life member and office bearer of many national societies, academies and associations. He has successfully completed a number of research/consultancy projects funded by various governments, private and multinational agencies. His current areas of interest are histopathology, toxicology, pre-clinical safety pharmacology and reproductive efficacy studies of laboratory animals.

He is also Editor-in-chief of the book series “Perspectives in Animal Ecology and Reproduction” a Daya Publications, New Delhi, India. The Editor-in-chief of the American Biographical Institute, USA, has appointed him as Consulting Editor of The Contemporary Who’s Who. Dr. Gupta recently also appointed as Nominee for the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Govt. of India).

Dr. Anil K. Verma, Ph.D., M.N.A.Sc., FLS, London (born 1963-) Sr. Grade Lecturer, Department of Zoology, Govt. College for Women (P.G.), Gandhi Nagar, Jammu, J&K State, did his M.Sc. in Zoology (1986) from University of Jammu, Jammu. He has undergone his M.Phil. (1988) and awarded first rank and Ph.D. (1993) in the field of animal reproduction at the same University and has published about 50 research papers and review articles in reputed journals and books. He is also a member Editorial Board of the book series “Advances in Fish and Wildlife: Ecology and Biology” a Daya Publications, New Delhi. In recognition of his standing in greater scientific community, the Board of Directors of the American Association for the advancement of science (AAAS) New York, Washington, has awarded membership to him. Recently the Linnaean Society of London, U.K. has awarded fellowship to him in October 2006 in recognition of his contribution towards the cultivation of Science in Natural History.

Dr Sushma Koul (born 1954-) obtained her Masters and Doctorate from University of Jammu, Jammu-India and is serving as Deputy Director in Indian Institute of Integrative Medicine (CSIR), Jammu. She has contributed more than 50 research papers and review articles, guided 4 Ph.D. students during her 30 years of Scientific career in the field of Medicinal Plant Biotechnology. She has visited University of Tubingen (Germany) under INSA-DAD exchange programme and has worked with Prof. E. Reinhard in Pharmazeutisches Institute of Tubingen University. Her main areas of research are micropropagation, bioreactor cultivation, conservation biology, adaptive biology and bioprospecting of bioactive molecules.
Chapter 8

Pieris brassicae as Laboratory of Synthesis of New Compounds with Biological Potential: Interaction with Brassica oleracea var. costata and Brassica rapa var. rapa.

David M. Pereira¹, Patrícia Valentão¹, Federico Ferreres², Rosa Seabra¹ and Paula B. Andrade¹*

¹REQUIMTE/Serviço de Farmacognosia, Faculdade de Farmácia, Universidade do Porto, R. Aníbal Cunha, 164, 4050-047 Porto, Portugal
²Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain

ABSTRACT

Interactions between insects and plants have been studied for a long time, showing beneficial and deleterious effects for both organisms. A general overview about the relationship between glucosinolates, flavonoids and insects will be presented. Several aspects of the complex interactions established by cabbage white butterfly (Pieris brassicae L.; Lepidoptera: Pieridae) are also considered. The larvae of this species constitute a frequent pest of some Brassica species, which are an important way of subsistence in several countries. As insects are unable to synthesize

* Corresponding Author: E-mail: pandrade@ff.up.pt.
phenolic compounds or their precursors, their presence in the different stages of *P. brassicae* life cycle can only arise from the food it has ingested. Deacylation, deglycosylation and sulphotating steps are known to be involved in the metabolic process. Thus, with this study we intend to demonstrate the possible use of *P. brassicae* as a source of compounds with interest for the health, unusual in nature and hard to be synthesized in the laboratory. Two examples will be presented: the sequestration of phenolics by *P. brassicae* larvae fed with tronchuda cabbage leaves (*Brassica oleracea* L. var. *costata* DC.) and kept without food for one hour, and the phenolic profiles of *P. brassicae* at different development stages (larvae, exuviae and butterfly), its excrements and its host plant *Brassica rapa* var. *rapa* L.

**Keywords:** *Brassica oleracea* var. *costata*, *Brassica oleracea* var. *rapa*, Flavonoids, Glucosinolates, Insect-plant interactions, *Pieris brassicae*.

**Introduction**

The role of plant chemistry in shaping plant-insect relationships is well recognized, with a close association of certain oligophagous insects with specific chemicals of their host plants (Renwick, 2002).

Large white butterfly *Pieris brassicae* L. (Lepidoptera: Pieridae), an insect whose larvae constitutes a frequent pest of some *Brassica* species, has a life cycle that lasts about 45 days from egg to adult. The larvae feed exclusively on crucifers (namely, cauliflower, cabbage, turnip, nasturtium and, more rarely, on red cabbage and radish), while adults feed on the nectar of several plants (Renwick, 2002; Muriel and Grez, 2002).

Herein, it will be presented a general overview about the relationship between glucosinolates, flavonoids and insects, with special emphasis on *P. brassicae*. Additionally, two examples of the interaction between this insect with two different host plants (*Brassica oleracea* L. var. *costata* DC. and *Brassica rapa* var. *rapa* L.) will be presented. These examples reveal that those interactions produced interesting bioactive compounds.

**Interactions Insect-Plant**

**Glucosinolates**

Glucosinolates (Figure 8.1) are β-thioglucoside N-hydroxysulfates [also known as (Z)-(or cis)-N-hydroximinosulfate esters or S-glucopyranosyl thiohydroximates], with a side chain (R) and a sulfur-linked β-d-glucopyranose moiety. The side chain (R) is characterized by a wide variety of chemical structures, which depends on the aminoacid precursor. So, they are subdivided into three classes: aliphatic, indolic and aromatic (Fahey *et al.*, 2001).

Glucosinolates have frequently been quoted as both a defense against generalist herbivores and an agent for host choice by specialist herbivores (Moyes *et al.*, 2000).

In what concerns to plant-insect interactions, the most important reaction of glucosinolates is their hydrolysis by the enzyme myrosinase, with the production of compounds, including
Isothiocyanates, referred as the mustard oils. Glucosinolates and these volatile compounds are largely responsible for the close association between crucifers and their specialist insect invaders. Isothiocyanates may serve to attract specialist insects to their hosts, whereas glucosinolates often trigger oviposition or feeding after an insect lands on the plant (Renwick, 2002). Once glucosinolates, or their hydrolysis products, are generally toxic to non-adapted insects, specialists have ways to prevent the potential toxicity of these compounds. This may occur by rapid excretion, glucosides hydrolysis, inhibition of hydrolysis, the action of protective enzymes, or by sequestering the glucosinolates (Schoonhoven et al., 1998). This association was already demonstrated in the specific case of Pieris sp. larvae (Renwick, 2002).

In a general way, sequestration of plant toxic compounds in herbivores is correlated with aposematic coloration and gregarious behaviour. Once larvae of P. brassicae present these characteristics, it was suggested that it sequester glucosinolates of their host plants. In opposition, Pieris rapae L. (Lepidoptera: Pieridae) are camouflaged and solitary, so there’s no expectation of occurrence of that sequestration. To test this hypothesis and to check the repeatability of a study that did report the presence of the glucosinolate sinigrin (Figure 8.2A) in P. brassicae, Müller et al. (2003) analysed the glucosinolate composition of larvae reared on three species of Brassicaceae (Sinapis alba, Brassica nigra and Barbarea stricta). Host plant glucosinolates were found only in traces or not at all in the larvae of P. rapae and of P. brassicae reared on S. alba, B. nigra or B. stricta. Thus, the larvae of both species do not sequester these secondary metabolites from their host plants. The results obtained by Aplin et al. (1975) indicating the accumulation of sinigrin in P. brassicae pupae wasn’t confirmed. Also, the authors didn’t find a correlation between glucosinolate sequestration and aposematism or gregariousness in the two Pieris spp.

The explanation for the presence of glucosinolates only in vestigial traces in larvae and faeces of both Pieris species, was their hydrolysis by myrosinase present in the ingested leaf tissue and/or metabolisation by the insect. Larvae feeding on S. alba excreted a progenitor of 4-hydroxybenzylcyanide (HBC) in the faeces. One possible justification for the detection of HBC after sulfatase treatment of

![Figure 8.2: Chemical Structures of Sinigrin (A) and Sinalbin (B)](image-url)
faecal extracts is that sinalbin, in the *Pieris* digestive tract or body, is hydrolysed into HBC and that enzymatic conversion of HBC into HBC-sulfate occurs subsequently. As Müller et al. (2003) referred, conjugation of phenols (case of sinalbin, Figure 8.2A) with sulfate is a detoxification path, increasing water solubility, which allows the excretion of a dietary compound, an example of the so called ‘Phase 2 Metabolism’ (Brattsten 1992).

Later, it was demonstrate by Agerbirk et al. (2006) that caterpillars of *P. rapae* convert 4-hydroxybenzylglucosinolate (sinalbin, Figure 8.2B) of brassicaceous plants into 4-hydroxybenzylcyanide sulfate (HBC sulfate), having 4-hydroxybenzylcyanide (HBC) as intermediate. This apparently serves as a detoxification process, as alternative formation of a mustard oil is avoided.

It would be interesting to assess the biological activity of these products of detoxification of glucosinolates by insects, namely by *P. brassicae*.

**Flavonoids**

Like glucosinolates, flavonoids can influence the feeding behaviour of larvae and oviposition of adult insects (van Loon et al., 2002).

Flavonoid uptake is relatively diffused in the Lepidoptera, namely in butterfly families as Papilionidae, Nymphalidae and Lycaenidae, in which they participate in wing pigmentation (Burghardt et al., 1997, 2001; Schittko et al., 1999). Actually, although most pigments are likely to be synthesised de novo during scale development in the pupa, others are secondary plant metabolites obtained from the larval diet once insects are incapable to synthesise flavonoids or their precursors (Knüttel and Fiedler, 2001). Several studies confirmed that flavonoids in insects arise from the diet (Harborne and Grayer, 1994; Burghardt et al., 1997; Schittko et al., 1999; Knüttel and Fiedler, 2001). So, flavonoid uptake and metabolization is effectively depending on the specific flavonoid pattern of host plants (Burghardt et al., 1997, 2001; Geuder et al., 1997; Schittko et al., 1999).

Flavonoids sequestered by the larvae are later biotransformed, retained and shifted to the wings during the late pupal stage (Geuder et al., 1997). The antioxidant capacity of flavonoids is well recognized (Ferreres et al., 2006; Vrchovská et al., 2006) and can play different roles in insects, working as antibiotic and antiviral (Harborne and Grayer, 1994).

Regarding flavonoid patterns, it was demonstrated that only part of the flavonoidic compounds of host plant are sequestered by larvae, with the uptaken flavonoids being object of several glycosylation reactions. In addition, butterflies belonging to the same species can show distinct flavonoid composition (related to the host plant used in the larval phases). Also, there is a tendency for female butterflies to be more rich in flavonoids than males (Burghardt et al., 2001).

According to the above mentioned, flavonoids in insects are effectively allied to the contents of flavonoids in their food (Burghardt et al., 2000). Larvae revealed a preference for sequestering and metabolising quercetin and kaempferol derivatives, the main flavonoids in the analysed plants. Other flavonoids like myricetin derivatives, flavones and isoflavonoids were mostly excreted (Burghardt et al., 2001).

As far as we know, there are only two studies concerning the sequestration of phenolic compounds by *P. brassicae* from *Brassica* leaves: one about flavonoids uptake by *P. brassicae* from *B. oleracea* var. *costata* (tronchuda cabbage) and another involving the phenolic compounds in *P. brassicae* reared on *B. rapa* var *rapa* (turnip leaves).
These studies can be important, attending to the fact that the larvae may accumulate and/or metabolize host plant constituents, namely complex flavonol glycosides (Ferreres et al., 2005, 2006), constituting a source of potential bioactive compounds not available in nature.

**Flavonoid Pattern of Larvae of *Pieris brassicae* Reared on *Brassica oleracea* var. *costata***

The flavonoid pattern of larvae of cabbage white butterfly (*P. brassicae*) (Figure 8.3) reared on the leaves of tronchuda cabbage was analyzed by HPLC-DAD-MS/MS-ESI, a highly advanced and valuable technique for the characterization of complex phenolic molecules (Ferreres et al., 2007a).

Wild *P. brassicae* larvae (fourth instar) and respective tronchuda cabbage external leaves (from three individuals with 45 days-old) host plants were collected on fields located in Samil, Bragança, northeastern Portugal.

The phenolic composition of the tronchuda cabbage external leaves had already been studied in a previous work (Ferreres et al., 2005). The composition of the host leaves, from which the larvae feed, revealed to be similar to that described before, being detected thirteen kaempferol derivatives (Table 8.1). The flavonoid profile obtained with *P. brassicae* (Figures 8.4 and 8.5) was then compared with that of the cabbage. Kaempferol-3-O-sophoroside-7-O-glucoside, kaempferol-3-O-sophoroside-7-O-sophoroside and kaempferol-3-O-sophoroside were the only compounds that the larvae and cabbage had in common.

Although the glycosylation pattern of the flavonols was the same in both extracts, it was observed that the flavonol 3-O-glycosides represented more than ca. 50 per cent in the larvae extract (Table 8.2), while they corresponded to ca. 12 per cent of their food plant (Table 8.1). This was ascribed to the metabolism of the flavonols glycosylated at 3 and 7 positions present on tronchuda cabbage, or to a higher efficiency of sequestration of flavonol-3-O-glycosides (Ferreres et al., 2007a).

Kaempferol-3-O-sophoroside was the most abundant flavonol glycoside derivative without acylation, corresponding to ca. 16 per cent of the total amount of phenolic compounds of the larvae (Table 8.2), while in tronchuda cabbage leaves it only represented ca. 5 per cent (Table 8.1). This difference can result from the metabolism of kaempferol-3-O-sophoroside-7-O-glucoside and its acylated derivatives, which are the most abundant compounds of tronchuda cabbage external leaves.
### Table 8.1: Phenolic Composition of *Brassica oleracea* var. *costata* External Leaves (Ferreres et al., 2007a)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 + Kaempferol-3-O-sophorotrioside-7-O-glucoside</td>
<td>7.6</td>
</tr>
<tr>
<td>22 Kaempferol-3-O-(methoxycaffeoyl/caffeoyl)-sophoroside-7-O-glucoside</td>
<td></td>
</tr>
<tr>
<td>2 Kaempferol-3-O-sophoroside-7-O-glucoside</td>
<td>22.9</td>
</tr>
<tr>
<td>23 Kaempferol-3-O-sophorotrioside-7-O-sophoroside</td>
<td>1.4</td>
</tr>
<tr>
<td>3 + Kaempferol-3-O-sophoroside-7-O-sophoroside</td>
<td>11.4</td>
</tr>
<tr>
<td>24 Kaempferol-3-O-tetraglucoside-7-O-sophoroside</td>
<td></td>
</tr>
<tr>
<td>25 Kaempferol-3-O-(sinapoyl/caffeoyl)-sophoroside-7-O-glucoside</td>
<td>17.1</td>
</tr>
<tr>
<td>26 Kaempferol-3-O-(feruloyl/caffeoyl)-sophoroside-7-O-glucoside</td>
<td>27.8</td>
</tr>
<tr>
<td>27 + Kaempferol-3-O-sophorotrioside</td>
<td>5.1</td>
</tr>
<tr>
<td>28 Kaempferol-3-O-(sinapoyl)-sophoroside</td>
<td></td>
</tr>
<tr>
<td>29 Kaempferol-3-O-(feruloyl)-sophorotrioside</td>
<td>0.4</td>
</tr>
<tr>
<td>30 Kaempferol-3-O-(feruloyl)-sophoroside</td>
<td>1.1</td>
</tr>
<tr>
<td>15 Kaempferol-3-O-sophoroside</td>
<td>5.2</td>
</tr>
</tbody>
</table>

### Table 8.2: Phenolic Composition of *P. brassicae* (Ferreres et al., 2007a)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Quercetin-3-O-sophoroside-7-O-glucoside</td>
<td>8.7</td>
</tr>
<tr>
<td>2 Kaempferol-3-O-sophoroside-7-O-glucoside</td>
<td>10.0</td>
</tr>
<tr>
<td>3 Kaempferol-3-O-sophoroside-7-O-sophoroside</td>
<td>6.6</td>
</tr>
<tr>
<td>4 Quercetin-3-O-(feruloyl)-triglucoside-7-O-glucoside</td>
<td>4.5</td>
</tr>
<tr>
<td>5 Kaempferol-3-O-(sinapoyl)-triglucoside-7-O-glucoside</td>
<td>5.0</td>
</tr>
<tr>
<td>6 Kaempferol-3-O-(feruloyl)-triglucoside-7-O-glucoside</td>
<td>5.6</td>
</tr>
<tr>
<td>7 Kaempferol-3-O-(p-coumaroyl)-triglucoside-7-O-glucoside</td>
<td>2.6</td>
</tr>
<tr>
<td>8 Kaempferol-3-O-(methoxycaffeoyl)-sophoroside-7-O-glucoside</td>
<td>0.5</td>
</tr>
<tr>
<td>9 Kaempferol-3-O-(caffeoyl)-sophoroside-7-O-glucoside</td>
<td>1.8</td>
</tr>
<tr>
<td>10 Quercetin-3-O-(p-coumaroyl)-sophoroside</td>
<td>3.4</td>
</tr>
<tr>
<td>11 Kaempferol-3-O-(p-coumaroyl)-(isomer)</td>
<td>3.3</td>
</tr>
<tr>
<td>12 Kaempferol-3-O-(p-coumaroyl)-sophoroside</td>
<td>13.4</td>
</tr>
<tr>
<td>13 + Kaempferol-3-O-(methoxycaffeoyl)-sophoroside</td>
<td>9.2</td>
</tr>
<tr>
<td>14 Quercetin-3-O-sophoroside</td>
<td></td>
</tr>
<tr>
<td>15 Kaempferol-3-O-sophoroside</td>
<td>15.8</td>
</tr>
<tr>
<td>16 Kaempferol-3-O-(p-coumaroyl)-sophoroside (isomer)</td>
<td>2.4</td>
</tr>
<tr>
<td>17 Kaempferol-3-O-(isinapoyl)-triglucoside-7-O-glucoside</td>
<td>2.1</td>
</tr>
<tr>
<td>18 Kaempferol-3-O-(feruloyl/sinapoyl)-triglucoside-7-O-glucoside</td>
<td>1.3</td>
</tr>
<tr>
<td>19 Quercetin-3-O-(feruloyl)-triglucoside</td>
<td>1.9</td>
</tr>
<tr>
<td>20 Kaempferol-3-O-glucoside</td>
<td>1.9</td>
</tr>
</tbody>
</table>
In what concerns the presence of quercetin derivatives, the authors mention that they have re-analysed the composition of the external leaves of tronchuda cabbage (Ferreres et al., 2005) and they had detected these compounds in vestigial amounts, which also happened with the tronchuda cabbage external leaves eaten by *P. brassicae*. The larvae contained high amounts of quercetin derivatives (ca. 18 per cent of the total amount of phenolic compounds) (Table 8.2), while in tronchuda cabbage these compounds were present only in trace amounts, which suggests that *P. brassicae* selectively sequesters these flavonoids or that the kaempferol glycosides are metabolised into quercetin glycosides by the larvae.

The presence of *p*-coumaroyl derivatives (kaempferol-3-\(O\)-(*p*-coumaroyl)-triglucoside-7-\(O\)-glucoside, quercetin-3-\(O\)-(*p*-coumaroyl)-sophoroside, kaempferol-3-\(O\)-(*p*-coumaroyl)–triglucoside, kaempferol-3-\(O\)-(*p*-coumaroyl)-sophoroside and respective isomer) in larvae aqueous extract (Figure 8.2), which had not been found on either the internal or external leaves of tronchuda cabbage (Ferreres et al., 2005; Sousa et al., 2005) were explained by the demethoxylation of the sinapoyl and/or *feruloyl*.

![Figure 8.4: HPLC-DAD Phenolic Profile of *Pieris brassicae* Larvae Hydromethanolic Extract, Reared with *B. oleracea* var. *costata.*](image-url)

Detection at 330 nm. Peaks: (1) quercetin-3-\(O\)-sophoroside-7-\(O\)-glucoside; (2) kaempferol-3-\(O\)-sophoroside-7-\(O\)-glucoside; (3) kaempferol-3-\(O\)-sophoroside-7-\(O\)-sophoroside; (4) quercetin-3-\(O\)-(*feruloyl*)-triglucoside-7-\(O\)-glucoside; (5) kaempferol-3-\(O\) (sinapoyl)-triglucoside-7-\(O\) glucoside; (6) kaempferol-3-\(O\) (*feruloyl*)-triglucoside-7-\(O\)-glucoside; (7) kaempferol-3-\(O\) (*p*-coumaroyl)-triglucoside-7-\(O\)-glucoside; (8) kaempferol-3-\(O\) (methoxycaffeoyl)-sophoroside-7-\(O\)-glucoside; (9) kaempferol-3-\(O\) (caffeoyl)-sophoroside-7-\(O\)-glucoside; (10) quercetin-3-\(O\) (*p*-coumaroyl)-sophoroside; (11) kaempferol-3-\(O\) (*p*-coumaroyl)–triglucoside; (12) kaempferol-3-\(O\) (*p*-coumaroyl)-sophoroside; (13) kaempferol-3-\(O\) (methoxycaffeoyl)-sophoroside; (14) quercetin-3-\(O\)-sophoroside; (15) kaempferol-3-\(O\)-sophoroside; (16) kaempferol-3-\(O\) (*p*-coumaroyl)-sophoroside (isomer); (17) kaempferol-3-\(O\) (disinapoyl)-triglucoside-7-\(O\)-glucoside; (18) kaempferol-3-\(O\) (*feruloyl/sinapoyl*)-triglucoside-7-\(O\)-glucoside; (19) quercetin-3-\(O\) (*feruloyl*)-triglucoside; (20) kaempferol-3-\(O\)-glucoside (Ferreres et al., 2007a).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R₁ = sophoroside; R₂ = glucoside</td>
</tr>
<tr>
<td>2</td>
<td>R₁ = sophoroside; R₂ = glucoside</td>
</tr>
<tr>
<td>3</td>
<td>R₁ = sophoroside; R₂ = sophoroside</td>
</tr>
<tr>
<td>4</td>
<td>R₁ = (feruloyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>5</td>
<td>R₁ = (sinapoyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>6</td>
<td>R₁ = (feruloyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>7</td>
<td>R₁ = (p-coumaroyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>8</td>
<td>R₁ = (methoxycaffeoyl)sophoroside; R₂ = glucoside</td>
</tr>
<tr>
<td>9</td>
<td>R₁ = (caffeoyl)sophoroside; R₂ = glucoside</td>
</tr>
<tr>
<td>10</td>
<td>R₁ = (p-coumaroyl)sophoroside; R₂ = H</td>
</tr>
<tr>
<td>11</td>
<td>R₁ = (p-coumaroyl)triglucoside; R₂ = H</td>
</tr>
<tr>
<td>12, 16</td>
<td>R₁ = (p-coumaroyl)sophoroside; R₂ = H</td>
</tr>
<tr>
<td>13</td>
<td>R₁ = (methoxycaffeoyl)sophoroside; R₂ = H</td>
</tr>
<tr>
<td>14</td>
<td>R₁ = sophoroside; R₂ = H</td>
</tr>
<tr>
<td>15</td>
<td>R₁ = sophoroside; R₂ = H</td>
</tr>
<tr>
<td>16</td>
<td>R₁ = (disinapoyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>17</td>
<td>R₁ = (feruloyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>18</td>
<td>R₁ = (methoxycaffeoyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>19</td>
<td>R₁ = (p-coumaroyl)sophoroside; R₂ = H</td>
</tr>
<tr>
<td>20</td>
<td>R₁ = (disinapoyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>21</td>
<td>R₁ = (methoxycaffeoyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>22</td>
<td>R₁ = sophorotrioside; R₂ = glucoside</td>
</tr>
<tr>
<td>23</td>
<td>R₁ = sophoroside; R₂ = sophoroside</td>
</tr>
<tr>
<td>24</td>
<td>R₁ = tetraglucoside; R₂ = sophoroside</td>
</tr>
<tr>
<td>25</td>
<td>R₁ = (sinapoyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>26</td>
<td>R₁ = (feruloyl/coumaroyl)triglucoside; R₂ = glucoside</td>
</tr>
<tr>
<td>27</td>
<td>R₁ = (sinapoyl)triglucoside; R₂ = H</td>
</tr>
<tr>
<td>28</td>
<td>R₁ = (feruloyl)triglucoside; R₂ = H</td>
</tr>
<tr>
<td>29</td>
<td>R₁ = (feruloyl)triglucoside; R₂ = H</td>
</tr>
<tr>
<td>30</td>
<td>R₁ = (feruloyl)triglucoside; R₂ = H</td>
</tr>
</tbody>
</table>

Figure 8.5: Structures of the Phenolic Compounds Identified in *Pieris brassicae* and *Brassica oleracea var. costata*. Identity of compounds as in Tables 8.1 and 8.2.

derivatives during the metabolism process in the larvae. On the other hand, Ferreres et al. (2007) referred that the absorbance of the peaks observed in Figure 4 for acylated flavonoid derivatives, could not be taken as proportional to their abundance, as some of them co-eluted with other unidentified cinnamoyl acids’ derivatives, presenting a similar UV spectrum and contributing to the overall absorbance of those peaks. Another advanced explanation was that, despite their existence in trochuda cabbage leaves in concentrations below the detection limits, *P. brassicae* selective uptakes and accumulates them.
The existence of two methoxylated flavonol derivatives in *P. brassicae* (kaempferol-3-O-(methoxycaffeoyl)-sophoroside-7-O-glucoside and kaempferol-3-O-(methoxycaffeoyl)-sophoroside) (Figure 8.4) were explained as a result from the metabolism of kaempferol-3-O-(methoxycaffeoyl/cofeooyl)-sophoroside-7-O-glucoside present on the external leaves of the tronchuda cabbage.

**Phenolics in *Pieris brassicae* Related on *Brassica rapa* var. *rapa***

The aim of the study of Ferreres et al. (2008) was to characterize the phenolic compounds of *B. rapa* var. *rapa* leaves and to establish possible relations with their ingestion, metabolism and accumulation by *P. brassicae* in the different stages of its life cycle (Figure 8.6). For this purpose, turnip leaves, *P. brassicae* larvae reared on this leaves and deprived of food for 12 hours, their excrements, exuviae and butterflies were analysed by HPLC-DAD-MS/MS-ESI.

In *B. rapa* var. *rapa* leaves it were characterized for all the acylated derivatives (methoxycaffeic, caffeic, sinapic, ferulic and *p*-coumaric acids) of quercetin-3-O-sophoroside-7-O-glucoside, kaempferol-3-O-sophoroside-7-O-glucoside and kaempferol-3-O-sophoroside (Figure 8.7). The kaempferol-3-O-sophoroside-7-O-glucoside derivatives are the most abundant ones (Figure 8.7B), while those of kaempferol-3-O-sophoroside were present in trace amounts. Quercetin-3-O-sophoroside-7-O-glucoside derivatives are found in considerable contents (Figure 8.7B). However, the decacylated glycosides were detected only in vestigial amounts in the saponified hydromethanolic extract (Figure 8.7A). This can be due to the alkaline decomposition, during the saponification process, of phenolic compounds with an *o*-dihydroxy group, resulting in the presence of quercetin derivatives in trace amounts while caffeic acid is not observed.

In general, this kind of acylated derivatives (Figures 8.7B and 8.10) are very common in Brassicacea (Llorach et al., 2003, Vallejo et al., 2004, Ferreres et al., 2005, 2006 and 2007b), and particularly in distinct *B. rapa* subspecies (Romani et al., 2006; Rochfort et al., 2006). On the other hand, the presence of flavonol-3,7-di-O-glucosides, namely non-acylated isorhamnetin-3,7-di-O-glucoside characterizes *B. rapa* (Fernandes et al., 2007; Romani et al., 2006; Rochfort et al., 2006) relatively to other *Brassica* species. Despite contributing to the organoleptic characteristics of the plant, these compounds may participate in the defence against external aggressions.

The two main phenolic compounds in excrements’ hydromethanolic extract (Figure 8.8) were ferulic and sinapic acids (Figure 8.10). Other compounds present in considerable amounts, and that have been found before in the native hydromethanolic extract of *B. rapa* var. *rapa* leaves, were kaempferol-3-O-sophoroside, isorhamnetin-3,7-di-O-glucoside and isorhamnetin-3-O-glucoside. Some of the acylated derivatives already described were also detected, in low or trace amounts: kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside, quercetin-3-O-(sinapoyl)sophoroside-7-O-glucoside, quercetin-3-O-(feruloyl)sophoroside-7-O-glucoside, quercetin-3-O-(p-coumaroyl)sophoroside-7-O-glucoside, kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside, kaempferol-3-O-(p-coumaroyl)sophoroside-7-O-glucoside and kaempferol-3-O-(p-coumaroyl)sophoroside. Other compounds that were not found in the native extract of the leaves were kaempferol-3-O-sophoroside-7-O-glucoside, described in the saponificated extract, quercetin-3-O-sophoroside, kaempferol-3-O-soporotrioside and three isomers of kaempferol-3-O-(p-coumaroyl)sophoroside. In the first part of the chromatogram (Figure 8.8) several flavonoids derivatives were detected and were identified as sulphate flavonoids: isorhamnetin-3,7-di-O-glucoside sulphate and monoglucosides (kaempferol-3-O-glucoside sulphate isomers and isorhamnetin-3-O-glucoside sulphate isomers). This kind of compounds is very usual in animals’ metabolic process. Considering the obtained results it was inferred that during the metabolic process of *P. brassicae* it occurs the deacylation of flavonoids, leading to the disappearance or decrease of...
Figure 8.6: *Pieris brassicae* Material Reared on *B. rapa* var. *rapa* Leaves

A: *B. rapa* var. *rapa* leaves;
B: *P. brassicae* larvae;
C: *P. brassicae* excrements;
D: *P. brassicae* exuviae;
E: *P. brassicae* butterfly.
Figure 8.7: HPLC-DAD Phenolic Profile of Brassica rapa var. rapa Leaves.
(A) saponified hydromethanolic extract and (B) native hydromethanolic extract.

Detection at 330 nm. Peaks: (1) quercetin-3-O-sophoroside-7-O-glucoside; (2) kaempferol-3-O-sophorotrioside-7-O-glucoside; (3) kaempferol-3-O-sophoroside-7-O-glucoside; (4) quercetin-3,7-di-O-glucoside; (5) p-coumaric acid; (6) kaempferol-3,7-di-O-glucoside; (7) isorhamnetin-3,7-di-O-glucoside; (8) ferulic acid; (9) sinapic acid; (10) kaempferol-7-O-glucoside; (11) kaempferol-7-O-glucoside; (12) kaempferol-3-O-glucoside; (13) isorhamnetin-3-O-glucoside; (14) quercetin-3-O-(methoxycaffeoyl)sophoroside-7-O-glucoside; (15) quercetin-3-O-(caffeoyl)sophoroside-7-O-glucoside; (16) kaempferol-3-O-(methoxycaffeoyl)sophoroside-7-O-glucoside; (17) kaempferol-3-O-(caffeoyl)sophoroside-7-O-glucoside; (18) quercetin-3-O-(sinapoyl)sophoroside-7-O-glucoside; (19) quercetin-3-O-(feruloyl)sophoroside-7-O-glucoside; (20) quercetin-3-O-(p-coumaroyl)sophoroside-7-O-glucoside; (21) kaempferol-3-O-(sinapoyl)sophoroside-7-O-glucoside; (22) kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside; (23) kaempferol-3-O-(p-coumaroyl)sophoroside-7-O-glucoside; (24) quercetin-3-O-(caffeoyl)sophoroside-7-O-glucoside (isomer); (25) kaempferol-3-O-(feruloyl)sophoroside-7-O-glucoside (isomer); (26) kaempferol-3-O-(methoxycaffeoyl)sophoroside; (27) kaempferol-3-O-(p-coumaroyl)sophoroside-7-O-glucoside (isomer); (28) kaempferol-3-O-(caffeoyl)sophoroside; (29) kaempferol-3-O-(sinapoyl)sophoroside; (30) kaempferol-3-O-(feruloyl)sophoroside; (31) kaempferol-3-O-(p-coumaroyl)sophoroside (Ferreres et al., 2008).

Acylated derivatives. Besides this, the absence of glycosilation in the 7 position in the majority of flavonoid sulphates, as well as in the remaining compounds, indicates the loss of the sugar in this position, together with the above mentioned deacylation, in the derivatives of kaempferol-3-O-sophoroside-7-O-glucoside and of quercetin-3-O-sophoroside-7-O-glucoside and in kaempferol-3-O-
sophorotrioside-7-O-glucoside and isorhamnetin-3,7-di-O-glucoside, to originate kaempferol-3-O-sophoroside, quercetin-3-O-sophoroside and isorhamnetin-3-O-glucoside, respectively. On the other hand, monoglycosilation of the majoraty of sulphate flavonoids points to a new deglycosilation process.

The hydromethanolic extract of *P. brassicae* larvae presented compounds found in the excrements: ferulic and sinapic acids and kaempferol-3-O-sophoroside (Figures 8.9 and 8.10). Vestigial amounts of quercetin-3-O-sophoroside and kaempferol-3-O-sophorotrioside, detected in the excrements too, were also identified. These compounds may contribute to protect the larvae from external aggressions, like light, undesirable environmental conditions, oxidative phenomena or microbial agents.
Figure 8.10: Chemical Structures of Several Phenolic Compounds Identified in *Pieris brassicae* Material and in the Host *Brassica rapa* var. *rapa* Leaves. Identity of compounds as in Figures 8.6–8.8.
Hydromethanolic extracts of exuviae and butterflies analysed by HPLC-MS revealed peaks in trace amounts, none of them corresponding to the studied phenolic compounds or possibly related with them. Excrements are produced only at the larval stage, being the material containing higher phenolics content (Figure 8.8). These results are not surprising, considering that phenolic compounds are sequestered and undergo metabolism, regarding their detoxification and excretion. If the compounds are excreted, then they won’t be present in the subsequent stages, and this maybe the reason for not finding them in the exuviae and butterflies.

In the previous work (Ferreres et al., 2007a) using B. oleracea var. costata as host plant, it was already possible to see that the larva can sequester and metabolize this type of compounds. The phenolics of these two Brassica species are different, so, the phenolic profile found for the larvae is also distinct form that observed before. This fact confirms the strong dependency on the phenolic pattern of the host plant. In addition, the study involving P. brassicae excrements, exuviae and butterflies (Ferreres et al., 2008), allowed to accomplish that, besides deacetylation and deglycosylation already reported (Ferreres et al., 2007a), sulphating reactions also occur in the metabolic process of the larvae, and that phenolics are mainly excreted and not transferred into the wings.

As insects are unable to synthesize phenolic compounds or their precursors, their presence in the different stages of P. brassicae life cycle can only arise from the food it has ingested, that is, from the complex flavonoid derivatives and free phenolic acids of B. rapa var. rapa leaves. So, the detection of this kind of compounds in the larvae indicates that it has the ability to sequester them. Additionally, the fact that both larvae and excrements exhibit phenolic compounds distinct from those of the host plant evidences that the larvae has the capacity to metabolize these phytochemicals, and to excrete them by the faeces, which included sulphate derivatives, reported for the first time. As these kind of flavonoids are known for their antioxidant potential (Ferreres et al., 2006 and 2007b; Vrchovská et al., 2006), in what concerns the obtainment of potential health promoting compounds, unusual in nature and of difficult laboratorial synthesis, P. brassicae (larvae, exuviae and butterfly) and its excrements may constitute a promising source.

Conclusion

The two examples presented herein showed that the P. brassicae larvae reared on B. oleracea var. costata and B. rapa var. rapa leaves their excrements, exuviae and butterflies presented new compounds, with complex chemical structures, impossible to be synthesized in the laboratory. These compounds are related with those from B. oleracea var. costata (Ferreres et al., 2005) and B. rapa var. rapa (Ferreres et al., 2008). These two species revealed high antioxidant capacity for which its phenolic compounds are responsible (Ferreres et al., 2005, 2006, 2007b). So, there’s a strong possibility that extracts of P. brassicae larvae reared B. oleracea var. costata and B. rapa var. rapa leaves, their excrements, exuviae and butterflies provide powerful natural antioxidants. If that happens, those extracts may be used by the pharmaceutical industry in antioxidantive formulations for prevention of free radicals-mediated diseases, or even as preservative of other oxidizable formulations. The same can be applied to cosmetic industry, for which it can be further used in anti-ageing formulations. Food industry may employ it to prevent the oxidation of its products, maintaining their quality and safety and extending their shelf-life, or to improve their nutritional value, by incorporating the extract in foodstuffs, thus increasing the dietary supply of antioxidants. In addition, it may constitute an economical advantage for B. oleracea var costata and B. rapa var. rapa producers, who have great losses caused by P. brassicae infestations.
Acknowledgements

The authors are grateful to Fundação para a Ciência e a Tecnologia (PTDC/AGR-AAM/64150/2006) for financial support of this work.

REFERENCES


