Morphological and Biochemical Diversity of Dalmatian Pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.)

Martina GRDIŠA (✉)
Klaudija CAROVIĆ-STANKO
Ivan KOLAK
Zlatko ŠATOVIĆ

Summary

Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trevir./ Sch. Bip.) is a perennial herbaceous plant belonging to the *Asteraceae* family. It is endemic to the East coast of the Adriatic Sea and its natural habitat extends from Italy to northern Albania and up in the mountainous regions of Croatia, Bosnia and Herzegovina and Montenegro. Pyrethrum flowers yield an important insecticide, the pyrethrin. Pyrethrin is mainly concentrated in oil glands on the surface of the seed inside the tightly packed flower head, but they can also be found in the other plant parts, however in much lower concentrations. The pyrethrin exist as a combination of six insecticide active ingredients: pyrethrin I, cinerin I, jasmolin I, pyrethrin II, cinerin II and jasmolin II, with pyrethrin I and pyrethrin II present in higher concentrations. Pyrethrin is non-toxic to mammals and other worm-blooded animals, it is unstable in light, oxygen, water and at elevated temperatures and therefore highly biodegradable. Due to the fact it is environmentally safe it is leading insecticide in organic farming systems. The most scientific work concerning Dalmatian pyrethrum was focused on its morphological and biochemical traits that are relevant in breeding. Breeding programmes are primarily focused on increasing the yield of pyrethrin per unit area. Relative to dry flower weight, flower heads contain the majority of the pyrethrin. Croatian wild populations contain approximately 0.60 to 0.79 %, while clones in breeding programmes of Australia and Kenya contain up to 3.0 % of pyrethrin.

Key words

pyrethrin; pest control; natural insecticides; insect repellent; organic farming

University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska cesta 25, 10000 Zagreb, Croatia
✉ e-mail: mgrdisa@agr.hr

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Introduction

Pyrethrin has highly unusual insecticidal activity, with the ability to control or repel numerous insect species, with little or no adverse environmental impact (Casida and Quistad, 1995). Its insecticidal properties have been known for centuries. It has a quick knock-down effect on a wide range of insect species (Bhat and Menary, 1984), causing paralysis within a few minutes. It acts as contact poison and affects the central nervous system of insects, by blocking their nervous functions. The insect's nervous impulses fail, causing them to die. In low concentrations, pyrethrin acts as a repellent, causing the insect to flee the source of the chemicals. It is non-toxic to humans and worm-blooded animals. In mammals, pyrethrin is readily broken down into inactive forms and passes from the body quickly. Additional advantages of pyrethrin are twofold: it is not persistent as it breaks down readily in sunlight and evolved insect resistance has not been recorded so far.

Dried and grounded flowers have been traditionally used in Croatian agriculture. They were also used as the remedy against fleas and lice, and burned to repel the flying insects.

Dalmatian pyrethrum was cultivated in Croatia from the end of 19th century. From 1930 the production gradually decreased, especially during the World War II. Commercial use of DDT (Dichloro-Diphenyl-Trichloroethane) chemical insecticides ended the production of Dalmatian pyrethrum in Croatia. Nevertheless, production expanded to the other parts of the world. Nowadays, it is cultivated in more than 10 countries: Kenya, Australia (mainly north-west coast of Tasmania), Tanzania, Rwanda, Ecuador, France, Chile etc.

According to the Article 16(3) (c) of European Union Regulation (EC) No. 834/2007 on Organic Production and Labeling of Organic Products, use of pyrethrin extracts as a plant protection products is permitted in organic farming. They are considered as environmentally friendly insecticides and widely used in agriculture, horticulture, store products (Silcox and Roth, 1995), households (Kennedy and Hamilton, 1995) and for the control of animal pests (Gerberg, 1995).

Taxonomy and distribution

Dalmatian pyrethrum (*Tanacetum cinerariifolium* /Trev./ Schultz Bip.) is herbaceous perennial herb, belonging to the *Asteraceae* (*Compositae*) family. The *Asteraceae* family is one of the largest angiosperm family and comprises around 1300 genera and 25 000 species (Bremer, 1994). Genus *Tanacetum* is distributed in Europe, temperate Asia, North Africa, and North America. Some species have been introduced into South Africa, South America, Australia and New Zealand. The genus contains 152 species, including those earlier considered to belong to the separate genus *Pyrethrum* Zinn. (Bremer and Humphries, 1993). The following species can be found in Europe (Tutin et al., 1964-1980):

1. *Tanacetum achilleifolium* (M.Bieb.) Sch.Bip., syn.: *Pyrethrum achilleifolium* M.Bieb. subsp. *wisockianum* Pacz, *Chrysanthemum achilleifolium* (M.Bieb.) Prodán, *Pyrethrum achilleifolium* M.Bieb. subsp. *achilleifolium, Pyrethrum achilleifolium* M.Bieb.
2. *Tanacetum annuum* L., syn.: *Chrysanthemum annuum* (L.) Hayek
3. *Tanacetum bipinnatum* (L.) Sch.Bip., syn.: *Pyrethrum bipinnatum* (L.) Willd, *Chrysanthemum bipinnatum* L.
4. *Tanacetum cinerariifolium* (Trevir.) Sch.Bip., syn.: *Pyrethrum cinerariifolium* Trevir., *Chrysanthemum cinerariifolium* (Trevir.) Vis.
5. *Tanacetum corymbosum* (L.) Sch.Bip., syn.: *Pyrethrum subcorymbosum* (Sch.Bip.) Schur, *Pyrethrum corymbiferum* (L.) Schrank, *Chrysanthemum corymbosum* L.
6. *Tanacetum corymbosum* (L.) Sch.Bip. subsp. *corymbosum,* syn.: *Leucanthemum corymbosum* (L.) Gren. & Godr., *Pyrethrum corymbosum* (L.) Scop., *Chrysanthemum corymbosum* L. subsp. *corymbosum*
7. *Tanacetum corymbosum* (L.) Sch.Bip. subsp. *clusii* (Fisch. ex Rchb.) Heywood, syn.: *Tanacetum subcorymbosum* (Schur) Sch.Bip., *Chrysanthemum subcorymbosum* Schur., *Pyrethrum clusii* Fisch. ex Rchb.
8. *Tanacetum funkii* Sch.Bip. ex Willk.
9. *Tanacetum macrophyllum* (Waldst. & Kit.) Sch.Bip., syn.: *Chrysanthemum macrophyllum* Waldst. & Kit., *Pyrethrum macrophyllum* (Waldst. & Kit.) Willd.
10. *Tanacetum microphyllum* DC.
11. *Tanacetum millefolium* (L.) Tzvelev, syn.: *Pyrethrum millefoliatum* (L.) Willd., *Tanacetum kittaryanum* (C.A.Mey.) Tzvelev, *Chrysanthemum millefoliatum* L.
12. *Tanacetum mucronulatum* (Hoffmanns. & Link) Heywood, syn.: *Pyrethrum mucronulatum* Hoffmanns. & Link, *Chrysanthemum mucronulatum* (Hoffmanns. & Link) Cout.
13. *Tanacetum odessanum* (Klokov) Tzvelev
14. *Tanacetum paczoskii* (Zefir.) Tzvelev
15. *Tanacetum parthenifolium* (Willd.) Sch.Bip. syn.: *Pyrethrum parthenifolium* Willd.
16. *Tanacetum parthenium* (L.) Sch.Bip., syn.: *Leucanthemum parthenium* (L.) Gren. & Godr., *Pyrethrum parthenium* (L.) Sm., *Chrysanthemum parthenium* (L.) Bernh., non (Lam.) Gaterau
17. *Tanacetum santolina* M.Winkl.
18. *Tanacetum sclerophyllum* (Krasch.) Tzvelev
19. *Tanacetum uralense* (Krasch.) Tzvelev
20. *Tanacetum vahlii* DC.
21. *Tanacetum willkommii* Sch.Bip.
22. *Tanacetum vulgare* L. syn.: *Chrysanthemum audiberti* (DC.) P.Fourn., *Chrysanthemum tanacetum* Karsch, non Vis., *Chrysanthemum vulgare* (L.) Bernh., non (Lam.) Gaterau, *Pyrethrum vulgare* (L.) Boiss., *Tanacetum audibertii* (Req.) DC.

In Croatia, the following species can be found: Dalmatian pyrethrum (*Tanacetum cinerariifolium*/Trevir./ Sch. Bip.), common tansy (*Tanacetum vulgare* L.), tansy daisy (*Tanacetum macrophyllum* /Waldst. & Kit./ Sch. Bip.), feverfew (*Tanacetum* *parthenium* /L./ Sch. Bip.) and corymbflower tansy (*Tanacetum corymbosum* /L./ Sch. Bip.) (Nikolić et al., 2009).

Wild populations of Dalmatian pyrethrum in Croatia are distributed on the Eastern sub- Mediterranean dry grasslands and abundant populations can be found in southern parts of Istria (Premantura), Kvarner islands (Krk, Cres, Lošinj), mountains Velebit and Biokovo, and along Dalmatian coastal region and its islands (Brač, Hvar, Biševo, Vis, Korčula, Lastovo, Mljet) (unpublished data).

History

The history of pyrethrum usually starts with the mention of identified species *Chrysanthemum cinerariaefolium* Vis. which was first recorded in Dalmatia (Visiani, 1847). Pharmacist Antun Drobac (1810-1882) from Dubrovnik, Croatia was the first to prove its insecticidal activity (Bakarić, 2005). Around 1854 Dalmatian pyrethrum was introduced into cultivation along Dalmatian coast. It was a very profitable production for local producers and it influenced the agricultural development of Dalmatian coast. Ground pyrethrum flowers could be found for sale by the name '*Dalmatian Insect Powder*' in most European pharmacies. From 1885 dried flowers were also exported to the USA. The primary use of dried pyrethrum flowers was the control of mosquitoes and body lice on humans and animals (Glynne-Jones, 2001). Pyrethrum products were available for use even though the chemistry of active ingredients was unknown. In 1924, German chemist Herman Staudinger (1881-1965) and Lavoslav Ružička (1887-1976), a Croatian scientist, winner of the 1939 Nobel Prize in Chemistry, identified chemical structure of the active pyrethrin ingredients, pyrethrin I and pyrethrin II (Coomber, 1948). Based on these discoveries it was possible to proceed with the synthesis of pyrethroids. Pyrethroids are manufactured chemicals that are very similar in structure to pyrethrin, but are often more toxic to insects, as well as to mammals, and last longer in the environment. Chemical structure of cinerin I and II was identified in 1945, while the chemical structure of jasmolin I and II was identified in 1966.

'Golden years' of pyrethrum production in Dalmatia were from 1910 until 1930. In that time, phylloxera (*Daktulosphaira vitifoliae* Fitch) destroyed many vineyards and peasants began cultivating Dalmatian pyrethrum in abandoned vineyards. Pyrethrum production saved many families from starvation and emigration. From 1930 to the beginning of the World War II production rapidly decreased and discovery of synthetic pesticide DDT significantly reduced pyrethrum production. In 1928, it was introduced to the British Colony of Kenya. Captain Gilbert Walker was the first settler to grow pyrethrum commercially in Kenya. He imported some seed from Dalmatia and planted it on his farm in the highland near Nakuru (Chandler, 1948). During the World War II Kenya produced large amounts of this natural insecticide to delouse allied armies (Mocatta, 2003). By the 1945 it became the world's main producer and it still holds the leading position. From 1964 to 1966 some attempts have been made to revitalize pyrethrum production in Dalmatia, unfortunately, it was not profitable, due to the fact that the price of imported pyrethrum extract was much lower than production costs.

Nowadays, Kenya is a major source of pyrethrum, accounting for more than 70 % of the global supply. Other larger producers of pyrethrum are Australia (Tasmania), Tanzania and Rwanda. The pyrethrum extracts are mainly exported to USA, Europe and Asian countries. According to FAOSTAT in 2003, 2004 and 2005 Kenya produced 8000 tons of dried pyrethrum flowers on the production area of 9000 hectares, while Tanzania produced 2500 tons on the production area of 13500 hectares. In the same period, Rwanda produced 1000 tons of dried pyrethrum flowers on the production area of 2200 hectares. On average, 3 to 4 kg of fresh flowers yield 1 kg of dried flowers. Around 250 kg of dried flowers are produced per hectare during the first year, increasing to 1000-1200 kg per hectare in the second and third year. After the third year yield declines.

Morphology

Dalmatian pyrethrum is a perennial herb, 30 to 100 cm high. The shoots branch a few times before terminating into a white, daisy-like flower heads. The inflorescence is composed from two types of sessile florets. The white petalled ray florets are placed on the margin and the yellow disc-florets are in the centre, and they are placed on a slightly convex receptacle (Brewer, 1968). Disc florets have both male and female organs, while the ray florets are female (Bhat, 1995). On a single flower head, florets are arranged in a form of dense spiral. The ray florets have a strap shaped corolla with three teeth at the end. The base of corolla is tubular and envelopes a bi-lobed cylindrical style which is placed in the center of the floret on the top of the ovary. Ovary is pentagonal and green calyx is attached to it. A single erect ovule stands in the ovary. The yellow corolla of the disc florets is also tubular. Disc florets are bisexual. Stamens are inserted on short stalks at the base of the inner surface of the corolla. At anthesis, the bi-lobed style grows through a tube formed by five anthers which are united along the edges. Green calyx is also attached to a pentagonal ovary. Pollen grains are tri-nucleate. The flowers open approximately one month after the first buds become visible. The flowering rhythm encourages crosspollination.

Fruits of pyrethrum are pale brown, cylindrical or semicuneiform achenes, with 5 to 7 ridges and glabrous and slightly lustrous surface (Bojnanský and Fargašová, 2007). The active ingredient of pyrethrum, the pyrethrin, is mainly located in tiny oil glands on the surface of the achenes.

Pollination

The insects, mostly *Hymenoptera*, carry out the pollination in pyrethrum. In order to collect pollen and nectar insects visit the inflorescence. Their path follows the development of the flower, from margin to the center, and through that process, they deposit foreign pollen on the styles of fully opened flowers. A discharge of the ripe pollen takes place under influence of the elongation process of the style. As soon as the pollen ripens, the style grows through the tube formed by the united edges of the anthers. The pollen reaches the receptive surfaces of the style with the help of the long hairs on the top of the lobes. Few hours after pollen discharge the style unfolds and is ready to accept foreign pollen. The germination percentage of the pollen decreases relatively rapidly after anthesis (the period during which a flower is fully open and functional). Since the individual floret discharges the ripe pollen before it unfolds the receptive surfaces of the style, the limited life of the pollen after anthesis reduces the chance for own pollen to germinate on styles of the same floret. Therefore the germination percentage of the own pollen by the time the style becomes receptive can be ignored. This is a well known mechanism to avoid self-pollination (Bremer, 1968). Another incompatibility system also operates with even higher efficiency- sporophytically determined system. (Brewer, 1974; Parlevliet, 1975; Bhat, 1995).

Within sporophytic incompatibility, the reaction of the pollen is linked to a trinucleate pollen grains and inhibition of germination or early tube growth. In Dalmatian pyrethrum, self-fertilization is prevented by inhibition of germination of the trinucleate pollen on the stigma. Despite the above described mechanism, the self-fertilization has been also reported (Brewer, 1968).

Genetics

The basic chromosome number in pyrethrum is 2n=2x=18 (MacDonald, 1995), but triploids (2n=3x=27) (MacDonald, 1995; Ottaro, 1977) and tetraploids (2n=4x=36) (MacDonald, 1995) have been also recognized. To produce a triploid plant with 27 chromosomes, the tetraploids must be produced first and then crossed with a diploid parent. In pyrethrum, tetraploids have been produced by treating the seed with 0.2 % colchicine for five days. Vegetative buds can also be treated with colchicine to double the chromosome number (Bhat, 1995).

Ottaro (1977) reported close relationship between triploidy and an increase in the expression of some morphological traits. The aim of study was to determine morphological characteristics that could be used for simple identification of diploid and triploid pyrethrum plants. Reliable distinguishing traits were: flower diameter, number of pollen germpores and number of stomata per unit area of the leaf. Flower weight, plant height and length of stomata were found to be less reliable traits. The results showed that triploid plants had significantly larger and heavier flowers than diploids. The triploids were also significantly higher than diploid plants but it was not found to be a reliable method for separating diploids from triploids, due to overlapping observed in the distribution of the two groups, unlike germpore count as well as number of stomata per unit area of the leaf.

Breeding

The most scientific work concerning Dalmatian pyrethrum has been focused on those morphological and biochemical traits that have been relevant in breeding, with the aim of increasing the yield of pyrethrin per unit area (Parlevliet, 1969, 1974, 1975; Bhat and Menary, 1986; Bhat, 1995).

Other important breeding objectives have been the development of nonlodging cultivars resistant to economically important pests and diseases, differing in flower maturity and adaptable to different environmental conditions. According to Bhat (1995), the most important traits in pyrethrum breeding are pyrethrin yield and quality, morphological traits (such as bush size, flower shape and size and plant height), resistance to lodging and synchronous flowering.

Pyrethrin yield and quality are determined by dry flower yield per unit area, pyrethrin content in the dry matter of the flowers and the ratio of pyrethrin I to pyrethrin II.

Dry flower yield depends on fresh flower yield and moisture content and it must be expressed on moisture free bases. Fresh flower yield depends on the weight and the number of flowers. It is believed that genotypes with higher flower number per plant produce high flower yield. Flower weight is determined as dry or fresh weight of 100 flowers. The samples must be collected at the same maturity and dried at the same nondegrading temperature (50 °C) (Ngugi and Ikadu, 1990). The ratio between pyrethrin I and pyrethrin II determines the quality of pyrethrum extract (Morris et al., 2006).

Bush size, flower shape and size and plant height are morphological traits important for the design and development of the appropriate harvesting equipment. Bush size shows a strong interaction with environmental factors such as temperature, soil type and moisture. Larger bushes usually yield more flowers.

Flower shape and size are usually determined by the diameter of the disc florets. Larger flowers are more attractive, but because of their mass, they might cause lodging in weaker plants. Another disadvantage of large flowers is that they take longer to dry and therefore increase production costs.

Plant height is measured as the length of the central flowering shoots from the ground to the level of the flowers. Tall and weak plants with heavy flowers tend to lodge which is undesirable characteristic. Lodging is determined as an angle at which the flowering shoots stand in the relation to the ground level (Bhat, 1995). Other different classifications concerning this trait have also been described. For example, depending upon whether the growth was dense (stems grew erect), open (stems inclined to spread) or very open (stems lay on the ground), Chamberlain and Clark (1947) used the terms "compact", "open" or "prostrate". Scales of 1 to 10 and 1 to 5 have also been used for scoring this trait, with 1 being the most susceptible to lodging (Parlevliet and Contant, 1970; Parlevliet, 1974). Number of flowers per stem along with flower size influences resistance to lodging. Resistance to lodging is required for easy picking as well as for mechanized harvest (Parlevliet, 1974). Harvest of pyrethrum flowers is determined by their maturity. Some authors consider flowers mature when ½ to ¾ disc florets are open (Bhat, 1995).

The relationships between morphological and chemical traits have been studied in order to facilitate selection, by enabling identification of genotypes with higher pyrethrin content without performing chemical analysis.

Pandita and Bhat (1986) have conducted a study on 100 randomly selected pyrethrum plants with the aim of finding out the correlation of pyrethrin content with floral traits. The results of the study showed that the pyrethrin content was significantly positively correlated with the width of the disc floret. They concluded that it is possible that more pyrethrin in the ovary induces a swelling of florets and therefore increase the flower width.

The investigations to determine the nature and magnitude of genotypic and phenotypic relations between various traits determining the pyrethrin yield have been conducted. Parlevliet (1974) has determined that quantity of pyrethrin harvested per hectare depends on fresh flower yield and dry matter content of the flowers. Fresh flower yield is correlated with the flower size, fresh weight of 100 flowers, number of flowers per stem and number of stems during the growing period. This investigation resulted with the conclusion that flower size is negatively correlated with dry matter content of the flowers and the flower yield, while the pyrethrin content had no correlation with flower yield and flower size. However, Parlevliet did not present the data concerning the magnitude or the nature of these relationships.

Bhat and Menary (1986) have also tested genotypic and phenotypic correlations in pyrethrum. Tested parameters of yield were pyrethrin content in dry matter of the flowers, dry flower yield, number of flowers per plant and 100 flowers dry weight. The results have shown that pyrethrin yield was correlated to four traits in following order: flower yield, pyrethrin content, number of flowers per plant and 100 flowers weight. The results also showed that flower yield depends more on the number of flowers per plant than on 100 flowers dry weight (Pandita and Bhat, 1984; Bhat and Menary, 1986; Singh et al., 1987). There was also a negative correlation between the number of flowers per plant and 100 flowers dry weight. Pyrethrin content did not show significant correlation with any character other than flowers yield (Bhat and Menary, 1986).

According to Singh et al. (1987), traits significantly correlated with flower yield were bush diameter and plant height. Bush diameter is positively correlated with number of flowers per plant and plant height. Between flower size (diameter) and flower yield (Pandita and Bhat, 1986; Singh et al., 1988) as well as between flower size (diameter) and number of flowers per plant negative correlation has been observed. Negative correlation between flower yield and pyrethrin content was reported by Kroll (1958).

The relationship between flower yield and lodging is also important in pyrethrum breeding. Parlevliet (1974) has observed that lodging was highly affected by flower size and the number of flowers per stem. He also reported minimal relationship between lodging and total flower yield. According to Bhat (1995), these results are opposite to the observations in the field, where it was shown that higher flower yield resulted with more lodging of the plants. Explanation of the conclusion made by Parlevliet is the fact that it was based on a presumption that the number of flowers per stem and flower yield were not related and therefore total flower yield and lodging were also unrelated. Another explanation of conclusion made by Parlevliet is the fact that in Kenya pyrethrum flowers are harvested every 2-3 weeks at their maturity and therefore the weight of the flowers on the stem is reduced (Bhat, 1995).

Parent-offspring correlations were found to be highly significant for number of flowers per plant, flower weight, pyrethrin content and yield, signifying the highly heritable nature of these traits (Singh et al., 1988).

Chemistry

Pyrethrin is mainly concentrated in the flower heads, namely 93.7 % of pyrethrin is accumulated in achenes, and minor quantities in disc florets (2.0 %), ray florets (2.6 %), and receptacles (2.6 %). The term pyrethrin is referred to the six insecticide active ingredients: pyrethrin I, cinerin I, jasmolin I, pyrethrin II, cinerin II and jasmolin II (Figure 1). Pyrethrin I, cinerin I and jasmolin I are closely related insecticidal esters of chrysanthemic acid, while pyrethrin II, cinerin II and jasmolin II are insecticidal esters of pyrethric acid. The three chrysanthemic acid esters are commonly referred as pyrethrins I, and pyrethric acid esters as pyrethrins II (Essig and Zao, 2001b). Among these compounds pyrethrin I and pyrethrin II are the most predominant and active (Casida and Quistad, 1995). Pyrethrin I alone is toxic, acting in minutes, while pyrethrin II has a high knock-down effect. Insects easily metabolize pyrethrin II and recover in a few hours. Nevertheless, combination of pyrethrin I and pyrethrin II has an outstanding effect on wide range of insect species (Winney, 1979). The relative amount of each ingredient depends on the particular plant type, geographical source and time of harvest (Glynne-Jones A., 2001). Ratio of pyrethrin I to pyrethrin II determines the quality of the pyrethrin extract. The insecticidal activity increases with the increased ratio, which varies between 0.9-1.3 (Maciver, 1995). As the flowers approach maturity, a decrease in pyrethrin I/II ratio has been reported. (Pattenden, 1970).

Gnadinger and Corl (1930) conducted a study to determine the relation between maturity of the flowers and pyrethrin content. The results showed that before the buds were formed, the roots, leaves and stems did not contain any pyrethrin. As the buds formed and developed, their pyrethrin content gradually increased. The last and the most mature samples contained four times the percentage of pyrethrin found in the unexpanded buds. The results indicated that the pyrethrin content depends on the maturity of the plant as well as on the development of the flowers.

Head (1966) conducted a study to determine the appropriate time for harvesting the crop. In his investigation, samples of flower heads were taken at different stages of maturity, ranging from closed bud stage to full flower maturity (flower heads dry and seed suitable for collection). The results have shown that flowers had maximum pyrethrin content when 3-4 rows of disc florets were open and that the pyrethrin yield did not significantly increase beyond this stage.

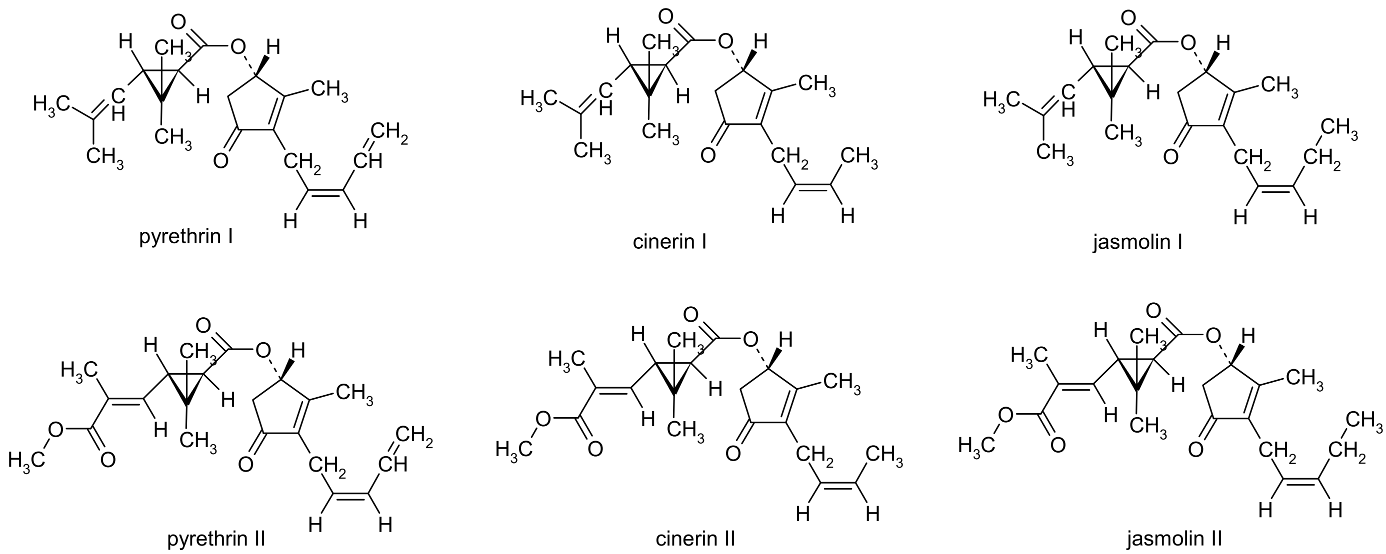

Figure 1. Chemical structure of pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmolin I and jasmolin II.

In Dalmatia, the flower heads were traditionally picked around June 13th (St. Anthony's Day). According to Bakarić (2005), flowers should be picked third or the fourth day after the blooming starts, i.e. when half of disc florets open. Pyrethrin content varies from 0.91 to 1.30 % of the dry flower weight (Kolak et al., 1999; Casida and Quistad, 1995). According to Bakarić (2005), wild Croatian populations of Dalmatian pyrethrum contain 0.60-0.79 % of pyrethrin. Parlevliet et al. (1979) analyzed twenty wild populations of Dalmatian pyrethrum for pyrethrin content. Wild populations were collected in Dalmatia and field trials were set on two locations in Kenya. The pyrethrin content ranged from 0.75 to 1.04 % (Bhat, 1995). Morris et al. (2005) reported for the Tasmanian commercial varieties to contain approximately 1.80 to 2.50 % of pyrethrin. According to Kiriamiti et al. (2003) in most cases pyrethrin content in the flowers ranges from 0.50 to 2.0% of the dry flower weight, while Pandita and Sharma (1990) reported for the pyrethrin content in the Dalmatian pyrethrum, bred and grown in India, to vary from 0.90 to 1.50 % of dried flower weight. In breeding programs of Australia, Kenya and the USA today, it is common to find clones with pyrethrin content of 3.0 % and more (Casida and Quistad, 1995).

Many investigations concerning the effect of drying air temperature and drying time on pyrethrin content have been conducted. Gnadinger (1936) reported that artificial drying at 50 °C does not affect pyrethrin content, but at 60 °C a slight loss has been observed and the loss increased at higher temperatures. The same results were obtained by Beckley (1952) and Ngugi and Ikadu (1990). Githinji (1973) conducted a series of drying experiments at 60 °C, 80 °C, 100 °C, 120 °C, 140°C and 160 °C, using a thin-layer of flowers. The best results were obtained while drying at 80 °C, with regular turning of the flowers, to avoid over drying of the flowers at the bottom of the layer. Shorter drying periods using higher temperatures were accompanied by the loss of pyrethrin. The loss of pyrethrin depended upon the air temperature and the duration of over drying.

Because of the difficulties concerning the separation of individual pyrethrin component and because of the lack of absolute standards for each component the content of pyrethrum extract is usually analyzed for total pyrethrin, or total pyrethrins I and total pyrethrins II. Many methods of pyrethrin extraction have been tested and used. The main objective of the extraction process is to obtain a light colored product, with a high recovery of pyrethrin active ingredients (Kiriamiti et al. 2003). The most commonly used methods of extraction in industry and laboratories are Soxhlet (SOX) (Otterbach and Wenclawiak, 1999), Ultrasonic (USE) (Kasaj et al., 1999) and recently the Supercritical Fluid Extraction (SFE) (Pan et al., 1995; Otterbach and Wenclawiack, 1999; Della Porta and Reverchon, 2002). It is of great importance to use suitable solvent for a particular extraction method. Many solvents have been used in pyrethrin extraction: propanol, ethanol, methanol (EU project, 2002), n-hexane (Pan et al., 1995, Kasaj et al., 1999, Kiriamiti et al., 2003), petrolether (Della Porta and Reverchon, 2002) and carbon dioxide (Kiriamiti et al., 2003). Three different extraction methods (USE, SOX and SFE) were compared for the extraction of pyrethrin. For USE the samples were extracted with 10 ml isopropanol, the SOX extraction was performed with 50 ml isopropanol in a micro-Soxhlet apparatus. The extraction conditions for SFE were a pressure of 30 MPa and temperature of 100 °C. To control the expansion of carbon dioxide, a heated (50 °C) regulated restrictor was used. Separation and determination of the samples was performed by Super Critical Fluid Chromatography (SFC- FID). The results have shown that the total amount of recovered pyrethrin in the solvents is very similar in all three extractions. Extraction time in USE and SOX is slightly higher (60 min) than in SFE extraction (20 min) (Otterbach and Wenclawiak, 1999). Wynn et al. (1995) described a preparative Supercritical carbon dioxide extraction process from pyrethrum flowers at 40 °C and 80 bars. The extraction efficiencies of supercritical carbon dioxide were much better than those of hexane. During the extraction process, the most efficient extraction period was the first three hours of the experiment. Kiriamiti et al. (2003) compared extraction of pyrethrin using supercritical carbon dioxide as a solvent with hexan Soxhlet extraction. The obtained extracts were very similar, whereas the only difference was in ratio of pyrethrin I to pyrethrin II, which was lower with supercritical carbon dioxide.

Many separation techniques have been compared and reported: Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) (Pan et al, 1995, Wang et al., 1997, Kasaj et al.,1999; Morris, 2006), Normal-Phase High-Performance Liquid Chromatography (NP-HPLC) (Essig and Zao, 2001a), Gas Chromatography (GC) (Nguyen et al., 1998) and some others. Due to thermal instability of pyrethrin, HPLC methods are preferred over GC methods for analyzing pyrethrin (Wang et al., 1997). Kasaj et al. (1999) have developed fast and precise RP-HPLC method for the quantification of total and individual amounts of the pyrethrin from pyrethrum extract and flowers by internal and external standardization. In the development of this method, the individual pyrethrins were isolated from the extract by preparative NP-HPLC. NP-HPLC is an analytical methodology, which provides the basis for quantitative analysis of each component in pyrethrum extract. It is usually used for the isolation of the individual compound while RP-HPLC is used for their quantitative determination (Kasaj et al., 1999). According to Wang et al. (1997), the advantage of RP-HPLC over NP-HPLC is very low level of interferences in the chromatography.

Conclusion

Insecticidal efficiency of pyrethrin has been known for centuries and the powder prepared from dried flowers has been used in traditional Croatian farming systems and household pest control. With the increase of production and consumption of healthy products as well as an increased resistance of pests to synthetic pesticides and more strict environmental legislation, interest in pyrethrin has been expanding continuously in recent years (Kolak, et al., 1999). Their very low toxicity to man and animals are ecological benefits which have led to increased production of this natural insecticide world-wide (Kasaj et al., 1999).

Formulations containing natural pyrethrin are widely used as household insecticides, for the control of pest in agriculture as well as for the prevention of infestations during storage. For the past 130 years of using pyrethrin on humans or near humans, the only complaints were due to the appearance of the skin allergies (Glynne-Jones, 2001).

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