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Alkaloids in *Achillea millefolium* L. — confusion in the literature

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Abstract

There is some confusion in the literature about alkaloids in *Achillea millefolium* L. (Asteraceae) and the purpose of this article is to clarify and correct this situation. In summary there are so far only four alkaloids that have been isolated from *A. millefolium*; betaine, betonicine (= achilleine), choline (or choline chloride) and stachydrine (= leonucardine). A fifth alkaloid, trigonelline, has been detected by paper chromatography but not isolated. A sixth, homostachydrine (moschatine or N-methyl piperidin-2-carboxylic acid methylbetaine), has not been isolated from *A. millefolium*, but from *A. moschata* WULF.

Keywords: *Achillea millefolium*, alkaloids, achiceine, achilleine, achilletine, betaine, betonicine, choline, homostachydrine, moschatine, stachydrine, trigonelline.

Introduction

Yarrow, *Achillea millefolium*, is a common medicinal plant that grows all over the northern hemisphere, primarily throughout the temperate and boreal zones. It has tradtionally been used for a variety of medical purposes, such as treating wounds and curing different kinds of stomach ailments[1]. Extracts from the plant have shown different types of pharmacological activites, including anti-inflammatory and antispasmodic effect[2]. However, in the literature that deals with the alkaloids of *A. millefolium*, there is some contradictory information. One review article lists some 8 alkaloids[1] but a more careful approach to the literature would indicate that this is an overestimation. The purpose of this brief survey is to correct the literature.

There are only four alkaloids that have been isolated from *Achillea millefolium*

[1]References, se page 4-5

according to the literature: betaine, betonicine, choline and stachydrine. Trigonelline has been detected by paper chromatography in a crude mixture. The structures of these compounds are shown in Fig. 1.

Survey of literature

The different studies dealing with alkaloids from *Achillea millefolium* are summarized in Table 1 which is arranged in chronological order.

One paper by Zirvi & Ikram[3] claims that *A. millefolium* contains the following alkaloids: achiceine ($C_{11}H_{17}NO_4$), achilletine (no formula presented), achilleine ($C_{14}H_{13}NO_3$), choline chloride ($C_5H_{14}NOCl$), homostachydrine ($C_{18}H_{15}NO_2$, this must be a misprint and should be $C_8H_{15}NO_2$) and stachydrine ($C_7H_{13}NO_2$). The same paper also reports that moscatine ($C_{21}H_{27}NO_7$) and trigonelline ($C_7H_7NO_2$) are constituents of *Achillea* species. According to a more recent review[1] *A. millefolium* contains: achiceine ($C_{11}H_{17}NO_4$), betaine ($C_5H_{11}NO_2$), betonicine ($C_7H_{13}NO_3$), choline ($C_5H_{14}NO$), homostachydrine ($C_8H_{15}NO_2$), moschatine (or moscatine) ($C_{21}H_{27}NO_7$), stachydrine ($C_7H_{13}NO_2$) and trigonelline ($C_7H_7NO_2$)

Many similarities are found in the reference lists of these authors. Zirvi & Ikram[3] have mainly used different kinds of handbooks and reviews[4-7] but also some original papers[8-9]. The handbooks and reviews are often referring to each other and have failed to consult the primary literature. Chandler et al.[1] on the other hand are using original papers[8-12] with two exceptions [3,7]. However, much of the material mentioned in Zirvi & Ikram[3] is neglected by Chandler et al.[1]

The first paper that describes isolation of an alkaloid from *A. millefolium* is Zanon[12], i.e. the "isolation" of achilleine with a yield of 6.5 %. von Planta-Reichenau[13] was the first to question whether this was a pure substance or not. That report also describes the isolation of two alkaloids from *A. moschata* and these were named achilleine (in German *achillein*) ($C_{20}H_{38}N_2O_{15}$) and moschatine (in German *moschatin*) ($C_{21}H_{27}NO_7$). When achilleine was hydrolyzed, it formed the alkaloid achilletine ($C_{11}H_{17}NO_4$), ammonia and a reducing sugar. This reaction is described in several alkaloid handbooks (i.e. 6, 14, 15, 16). In 1928 H. Schaller in Zürich explained that he could not show the presence of achilleine in *A. millefolium* or *A. moschata* in spite of repeated experiments[15]. According to the review article by Zirvi & Ikram[3] both achilleine and achilletine are constituents of *A. millefolium* and moschatine is considered to occur only in *A. moschata*, but this is only based on literature data from different handbooks.

In the 1950s Miller & Chow[8] isolated on alkaloid from *A. millefolium* and named it

achilleine, having the formula $C_{14}H_{26}N_2O_6$. The formula was later revised when PAILER & KUMP[11] isolated an alkaloid with the same composition judged from the elemental analysis buth with only half the molecular weight. In that paper the alkaloid was studied both chemically and by infrared-spectroscopy and it was judged that achilleine was identical to betonicine ($C_7H_{13}NO_3$).

PAILER & KUMP[9] isolated further alkaloids from both *A. millefolium* (betaine [$C_5H_{11}NO_2$], choline [$C_5H_{14}NO$] and stachydrine [$C_7H_{13}NO_2$]) and *A. moschata* (homostachydrine [$C_8H_{15}NO_2$; N-methyl piperidin-2-carboxylic acid methylbetaine]). Homostachydrine is later called moschatine by HEGNAUER[5]. Homostachydrine is also mentioned in KARRER[17]: "L(-)-Homostachydrin ... aus Kraut von *Achillea millefolium* L. und *A. moschata* WULF. isoliert" [L(-)-Homostachydrine ... isolated from the herbs of *Achillea millefolium* L. and *A. moschata* WULF.] with PAILER & KUMP[9] as reference. Merck Index[18] from 1989 does not have any entries on achiceine, achilleine, achilletine, homostachydrine or moschatine; only betonicine is mentioned in connection with *A. millefolium.*

A subsequent study on the alkaloid content in *A. millefolium* was made by IVANOV & YANKOV[10] who used an ion exchanger for a crude separation and paper chromatography with reference compounds for the analysis. They managed to detect the already reported alkaloids: betaine, betonicine, choline, stachydrine and another alkaloid with an Rf value similar to trigonelline.

Both CHANDLER et al.[1] and ZIRVI & IKRAM[3] refer to an alkaloid named achiceine. CHANDLER et al. have used ZIRVI & IKRAM[3] and SOKOLOV[7] as sources while ZIRVI & IKRAM only used SOKOLOV. SOKOLOV has on the other hand used two different sources[19,20] written in Russian and these two references have not been available to me. However, the most recent source[19] from 1939 is described in Chemical Abstracts as a screening for alkaloid content in 259 different plants and no isolation work is mentioned. SOKOLOV[7] writes that *A. millefolium* "contains the glucoalkaloid achilleine $C_{20}H_{38}N_2O_{15}$ and the amorphous basic achiceine $C_{11}H_{17}NO_4$". The alkaloids isolated by ZANON[12] and VON PLANTA-REICHENAU[13] achiceine and achilletine are described as amorphous bases which have the same molecular formula as the compounds described by SOKOLOV. It might be possible that SOKOLOV's two sources, LAZUREVSKII & SADYKOV[19] and SHACKII[20] are quoting ZANON and VON PLANTA-REICHENAU, but this, at present is uncertain.

A more recent study demonstrated, by TLC-comparison with reference compounds and the use of Dragendorff spray reagent, the presence of betaine, betonicine, sholine, stachydrine and trigonelline in *A. millefolium*, while homostachydrine could not be detected[21]. *A. millefolium* is now considered to be a group of several closely related

species and a very recent article described the pattern of different betaines in species of the aggregate. They investigated the presence of betaine, betonicine, choline, and stachydrine in 46 individuals from 11 species of the *A. millefolium* group[22].

However, some of the earlier papers are still quoted in scientific studies. Recently errors concerning the alkaloids from *A. millefolium* appeared in different papers[23-24]. ETMAN et al.[23] have studied *A. santolina* and have performed a microanalysis on an isolated alkaloid. They determined the formula $C_{14}H_{26}N_2O_6$ and reported that it was "an expected formula similar to that of the alkaloid isolated from *Achillea millefolium*, named Achilleine (MILLER & CHOW 1954). Therefore, it could be suggested that the separated alkaloid in this work from *Achillea santolina* may be achilleine"[23]. PAILER & KUMP[11] showed that achilleine was identical with betonicine, $C_7H_{13}NO_3$.

Conclusion

In conclusion the alkaloids that have been found in *A. millefolium* are until now: betaine, betonicine, choline, stachydrine and probably trigonelline. All the other alkaloids mentioned in the literature have either been isolated from other species (e.g. *A. moschata*) or are synonyms for some of the above mentioned alkaloids. Some of the earliest reported alkaloids might have been crude fractions and not pure compounds.

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Table 1. A summary of the literature dealing with the betaine-type alkaloids from *Achillea millefolium*.

Achilleine isolated from *A. millefolium* (ZANON 1846)[12].

Claimed that ZANON's achilleine was not a pure substance and isolated two alkaloids from *A. moschata*, named **achilleine** ($C_{20}H_{38}N_2O_{15}$) and moschatine ($C_{21}H_{27}NO_7$). By hydrolysis of **achilleine** an alkaloid named **achilletine** ($C_{11}H_{17}NO_4$) is formed (VON PLANTA-REICHENAU 1870)[13].

Failed to show the presence of **achilleine** in *A. millefolium* or *A. moschata* (SCHALLER 1928)[15].

Literature-review. *A. millefolium* contains **achilleine** ($C_{20}H_{38}N_2O_{15}$) and **achiceine** ($C_{11}H_{17}NO_4$) (SOKOLOV 1952)[7].

Isolated **achilleine** ($C_{14}H_{26}N_2O_6$) from *A. millefolium* (MILLER & CHOW 1954)[8].

Isolated **achilleine** and find that the formula is ($C_7H_{13}NO_3$). Spectroscopical studies show that it is identical with **betonicine** (PAILER & KUMP 1959)[11].

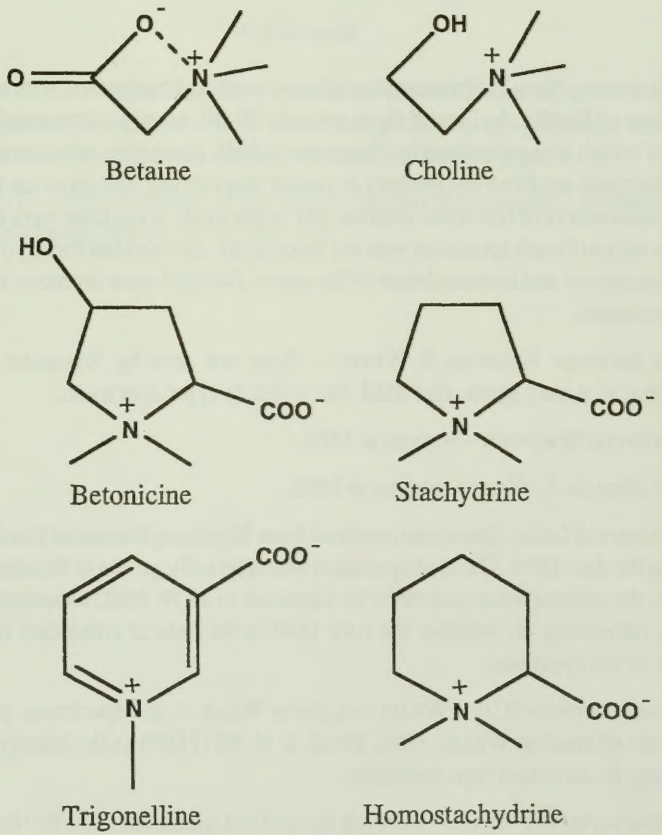
Betaine ($C_5H_{11}NO_2$), **choline** ($C_4H_{14}NO$) and **stachydrine** ($C_7H_{13}NO_2$) from *A. millefolium* and **homostachydrine** ($C_8H_{15}NO_2$) from *A. moschata* (PAILER & KUMP 1960)[9].

Homostachydrine is named **moschatine** but still isolated from *A. moschata* (PAILER & KUMP 1960)[9] according to HEGNAUER (1964)[5].

Detected **betaine, betonicine, choline, stachydrine** and a new alkaloid **trigonelline** ($C_7H_7NO_2$) (IVANOV & YANKOV 1971)[10].

Literature review. Claims that *A. millefolium* contains achiceine (SOKOLOV 1952)[7], **achilleine** (MILLER & CHOW 1954)[8], **achilletine, betonicine, choline chloride, stachydrine,** (Handbooks), **moscatine** and trigonelline in *Achillea* sp. (Handbooks) according to CHANDLER et al. (1982)[1].

Fig. 1. The structures of the alkaloids definitively isolated from or identified in *Achillea millefolium* and homostachydrine isolated from *A. moschata.*



Notice of type specimens of *Dahlia* Cav. in the National Herbarium of Victoria (MEL)

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Prior to moving from Melbourne to northern Australia I had reason to examine MEL's collection of *Dahlia*. As I recall there are only 20-30, mostly unmounted, specimens, none of which was examined by Sorensen (1969) during his revision of the genus. All collections are from last century or earlier this century and some are undoubtedly type specimens or of historical interest. My notes made at the time were not elaborate and the status of each specimen was not thoroughly checked but for anyone interested in the taxonomy and nomenclature of the genus *Dahlia* I draw attention to the following specimens.

Dahlia barkerae Knowles & Westc. – Type not seen by Sorensen. I recorded, surprising as it may seem, that MEL has a 'likely type' specimen.

Dahlia brevis Sorensen – isotype at MEL.

Dahlia dissecta S. Wats. – isotype at MEL.

Dahlia merckii Lehm. Specimen received from Hamburg Botanical Garden, the label bearing the date 1844. The type specimen was originally grown in the aforementioned garden, the species being described by Lehmann in 1839. MEL's specimen is perhaps a type, depending on whether the date 1844 is the date of collection or the date of receipt of the specimen.

Georgina coccinea (Cav.) Willd. var. *flava* Willd. – old specimen, perhaps type. Sorensen referred to Willd., Hort. Berol. 2: pl. 96 (1809) as the lecotype specimen. This may be an extant type specimen.

Georgina variabilis Willd. – there are several old specimens, one ex 'herbario Dr H. van Hemck', with this name. One specimen is labelled as var. *purpurea* Willd. Sorensen made reference to published plates as being the lectotype specimens of formally named varieties of *G. variabilis*. There is a possibility that there are extant types at MEL.

The presence of the *Dahlia* specimens, at least those obtained last century from Germany, is undoubtedly a result of the connections and efforts of the renowned botanist, FERDINAND MUELLER. During his term as Government Botanist of Victoria, a position he held from 1853 to 1896, MUELLER was responsible for the acquisition of a number of private herbaria, including those amassed by OTTO WILHELM SONDER and JOACHIM STEETZ (SHORT 1990), and as such MEL often houses important collections which may be overlooked by taxonomists.

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Chromosome studies on *Vernonia flexuosa* and *V. lithospermifolia*

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Abstract

Meiotic and mitotic chromosomes of *Vernonia flexuosa* Sims and *V. lithospermifolia* Hieron. were analyzed in detail. *V. flexuosa* presented 2n=4x=40 (28m + 12sm) and *V. lithospermifolia* showed 2n=4x=20 (10m + 2m-sm + 8sm). Meiotic behaviour was regular in both species and supports an allotetraploid origin of *V. flexuosa*. The results obtained in this work could contribute to a better taxonomic identification of the two species.

Introduction

Vernonia flexuosa and *V. lithospermifolia* are two closely related species distributed in Brazil, Paraguay, Argentina and Uruguay. The two species are morphologically similar, differing mainly in the head size and the leaf disposition and shape (Cabrera & Klein 1980). They are easily distinguished from other species of the genus by the combination of seriate-cymose inflorescence, sessile heads, yellowish-brownish pubescence on leaves and stems, and anther appendages with glands.

The two species belong to Cabrera's (1944) subsect. *Flexuosae*, or maybe better to genus *Chrysolaena* of Robinson (1988), which includes seven other taxa from South America, centralized geographically in southern Brazil and northern Argentina.

The cytology of this natural group is relatively well known. All the species have the basic chromosome number x=10, contrasting with most of related New World taxa, which commonly present x=17 or x=16 (Dematteis 1997). Previous studies have reported the chromosome number of *V. lithospermifolia* (Dematteis 1998) and described the karyotype of *V. flexuosa* (Ruas et al. 1991). However, to date no comparative study of the somatic and meiotic chromosomes of both entities has been made.

In this paper *V. flexuosa* and *V. lithospermifolia* are analyzed cytologically in detail with the purpose to provide data for an accurate identification of the two species.

Materials and methods

Voucher specimens were deposited in the herbarium of the Instituto de Botánica del Nordeste (CTES). The material examined is as follows.

V. flexuosa Sims: **Uruguay. Dept. Artigas.** Tomás Gomensoro. Dematteis et al. 491 (CTES).

V. lithospermifolia Hieron.: **Argentina. Corrientes. Dept. Saladas.** Río San Lorenzo. Dematteis & Solís Neffa 503 (CTES, LP).

Chromosome studies were made from root tips of germinating seeds. After a pretreatment of about 4 hours in 8-hydroxyquinoline 0,002 M, the roots were fixed in ethanol-acetic acid (3:1) and stained according to the Feulgen technique. Nomenclature used for karyotype description is that proposed by Levan et al. (1964). Chromosome morphology was characterized using the centromeric index (ci = short arm x 100/total chromosome length). The idiograms and measures were obtained from the mean of ten metaphase plates for each species.

The fertility and diameter of the pollen grains were estimated from herbarium specimens by stain with carmin-glicerina (1:1).

Results

Chromosome number, total chromosome length, mean chromosome length, centromeric index and pollen diameter of *V. flexuosa* and *V. lithospermifolia* are summarized in Table 1.

The somatic chromosome number of *V. flexuosa* was found to be 2n=40 (Fig. 1). Karyotype was composed of 28m + 12sm (Fig. 3). Chromosomes ranged in length from 1,50 to 3,20 µm. The pair 2m showed a macrosatellite in the short arm, while the pair 12m presented a microsatellite in the short arm.

In *V. lithospermifolia* 2n=20 chromosomes were observed (Fig. 2). The karyotype formula was composed of 10m + 2m-sm + 8sm (Fig. 4). The pair 3m showed macrosatellite in the long arm and the pair 6m presented microsatellite in the short arm.

Meiotic behaviour was regular in the two species, showing always bivalents, 20II in *V. flexuosa* and 10II in *V. lithospermifolia.* Fertility of pollen grains was 95,30 % in *V. flexuosa* and 96,36 % in *V. lithospermifolia.* Results indicate that *V. lithospermifolia* is diploid on base x=10, while *V. flexuosa* in view of its meiotic behaviour would be considered allotetraploid with the same basic chromosome number.

Discussion

Most taxonomic treatments on the *Flexuosae* group of *Vernonia* distinguish *V. flexuosa* and *V. lithospermifolia* according to the head size, number of flowers, and leaf shape (Cabrera & Klein 1980, Jones 1981, Robinson 1988). However, in several cases it is difficult to make a clear distinction between the species, due to the wide variation and apparent overlapping in these characters.

V. flexuosa shows a great variation in head size, ranging from (6) 7 to 12 (14) mm in length. This was first noted by Hieronymus (1897), who established four varieties according to this feature. *V. lithospermifolia* is particularly similar to *V. flexuosa* var. *microcephala* Hieron., with which it may be confused due to the reduced heads of the latter taxon.

Results obtained here indicate that it is possible to perform a further distinction of these species considering the cytological information. From this viewpoint, *V. flexuosa* and *V. lithospermifolia* are certainly very distinct. Besides the evident difference in ploidy level, the karyotypes differ in number of metacentrics and submetacentrics, symmetry level and total length per haploid genome.

The difference in ploidy level between the species is also evident in the diameter of the pollen grains, which is relatively constant within each species. The pollen size might be effectively used to separate herbarium material that cannot be distinguished otherwise.

Despite the differences noted above, the two species have the same number and morphology of satellites. Most members of subsect. *Flexuosae* have constantly one or two chromosome pairs with a microsatellite (Dematteis 1997). *V. flexuosa* and *V. lithospermifolia* are the only two taxa of this group with both macro- and microsatellites, which supports the close relationship between these species.

Acknowledgements

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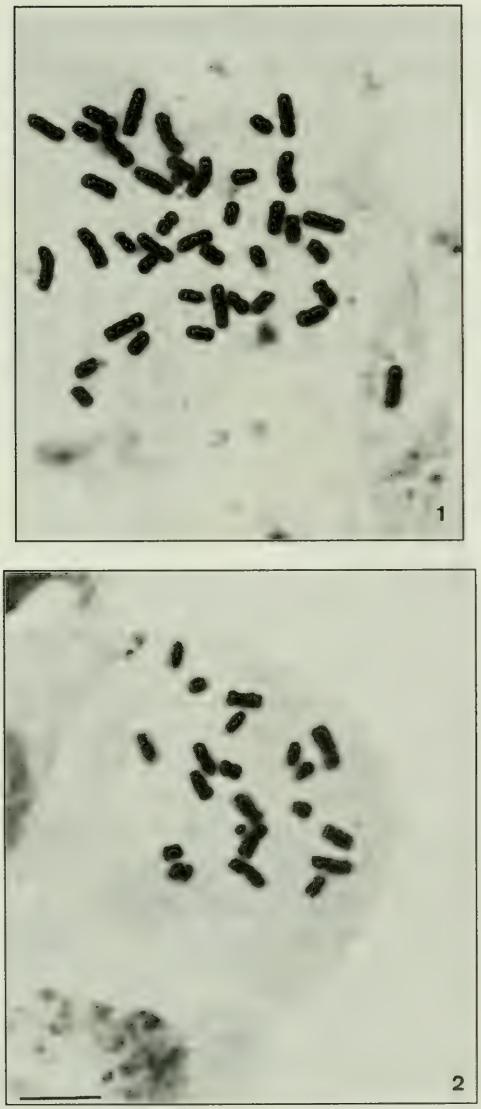
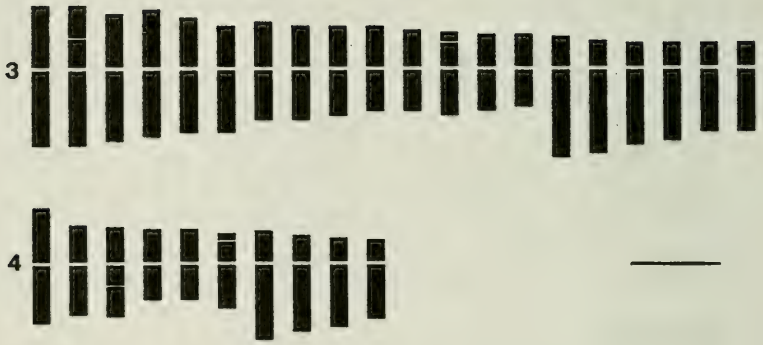
Table 1. Somatic chromosome number (2n), total chromosome length (TCL), mean chromosome length (ML), centromeric index (CI), and pollen diameter in µm (ø) of *V. flexuosa* and *V. lithospermifolia*.

Species	2n	TCL	ML	CI	ø pollen
V. flexuosa	40	94.60 ± 3.70	2.36	41.10 ± 0.50	(39) 41–42 (46)
V. lithospermifolia	20	40.54 ± 2.30	2.02	38.88 ± 0.23	(31) 32–33 (36)



Figs. 1-2. Somatic chromosomes of *V. flexuosa,* 2n=40 (1), and *V. lithospermifolia,* 2n=20 (2). Scale = 5 μm.



Figs. 3-4. Idiograms of *V. flexuosa*, 28m + 12sm (3) and *V. lithospermifolia*, 10m +2m-sm + 8sm (4). Scale = 2μm.

New names and combinations and other nomenclatural notes for Compositae of various countries

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Abstract

The author proposes new names, or the proper synonym, for taxa of different countries that hold the same names, new combinations in proper genera, and emphasizes older priorities on names currently used.

Introduction

The recent publication of floras of different countries has shown that there are some Compositae species holding the same name. Many of these are taxa with a narrow distribution, and the lack of available synonyms necessitates some new names to be established. Concerning other species, mainly of the Middle East or Mediterranean Region, the appropriate combinations, following the latest taxonomic treatments (e.g. Bremer 1994), have been traced. Furthermore, some combinations are reassigned to authors earlier than those currently cited.

Abbreviations: FC = Flora Vascular de Chile (Marticorena & Quezada 1985), FE = Flora Europaea (Tutin et al. 1976), FF = Index Synonimique de la Flore de France (Kerguélen 1993), FI= Flora d'Italia (Pignatti 1982), FO = Conspectus Florae Orientalis (Heller & Heyn 1993), FR = Vascular Plants of Russia (Czerepanov 1995), FSA = Plants of Southern Africa (Arnold & de Wet 1993).

New or correct names

Centaurea aksamica Soldano, nom. nov. = *C. veneris* B. L. Burtt et P. H. Davis, Kew Bull. 4: 104-105 (1949), non (Sommier) Béguinot in Béguinot et Landi, Arch. Bot. (Forlì) 7: 90 (1931).

Ref.: FO, 8:120.

The same name of this Cyprus endemite was earlier employed for another restricted taxon of NW Italy (Liguria) listed in FI.

Senecio incertus DC., Prodr. 6: 433 (1838) = *S. tuberosus* (DC.) HARV. in HARV. et SOND., Fl. Cap. 3: 375 (1865), non SCH. BIP. ex A. RICH., Tent. Fl. Abyss. 1: 434 (1848).

Ref.: FSA, 768.

The synonym of HARVEY's illegitimate combination that replaces it, is listed in the indicated reference. SCHULTZ BIPONTINUS' name validated by A. RICHARD concerns a species recently included in *Solanecio* as *S. tuberosus* (SCH. BIP. ex A. RICH.) C. JEFFREY (1986).

Senecio linaresensis SOLDANO, nom. nov. = *S. subdentatus* PHIL., Linnaea 28: 748 (1856), non LEDEB., Fl. Alt. 4: 110 (1837).

Ref.: FC, 39.

PHILIPPI's plant is an endemite of "Cordillera de Linares" in Chile and the LEDEBOUR one, currently listed (FR, 98), grows from Russian Caucasus to Eastern Siberia.

Senecio neoviscidulus SOLDANO, nom. nov. = *S. viscidulus* COMPTON, Journ. S. Afr. Bot. 33: 303 (1967), non SCHEELE, Linnaea 18: 480 (1844).

Ref.: FSA, 768.

COMPTON's species, an endemite of Natal and Swaziland, has the same name as the hybrid between *S. sylvaticus* L. and *S. viscosus* L., a taxon of Central-North Western Europe (BENOIT et al. 1975) listed, for example, in the latest British Flora (STACE 1995).

Senecio nublensis SOLDANO, nom. nov. = *S. carnosus* PHIL., Anal. Univ. Chile 21: 382 (1862), non THUNB., Prodr. Pl. Cap.: 158 (1800).

Ref.: FC, 38.

This endemite of Chillan Mountains in Chile (CABRERA 1949: 190-191) had the same binomial as the earlier THUNBERG taxon, a species restricted to South Africa, listed in FSA. Hovewer, it has also to be considered that THUNBERG's name is a later homonym of *S. carnosus* LAMARCK (1779) and it is therefore illegitimate (art. 53.1 of the Code of Nomenclature; GREUTER et al. 1994); but art. 56 of the same Code may be used (a proposal is in preparation) for the rejection of LAMARCK's name, considering the long permanence of THUNBERG's taxon in the literature and the illegitimacy of LAMARCK's name, a superfluous renaming of *Senecio doria* L.

Senecio pemehuensis SOLDANO, nom. nov. = *S. scoparius* PHIL., Anal. Univ. Chile 88: 282 (1894), non HARV. in HARV. et SOND. Fl. Cap. 3: 389 (1865).

Ref.: FC, 39.

HARVEY's earlier name concerns a South African endemite currently listed (FSA, 767). PHILIPPI's taxon is known only from a collection near Pemehue in south Chile (CABRERA 1949).

Senecio variifolius DC., Prodr. 6: 393 (1838) = *S. lyratus* L. fil., Suppl. 369 ("1781", publ. 1782), non FORSSK., Fl. Aeg. Arab. 148 (1775).

Ref.: FSA, 765.

FORSSKÅL's earlier name refers to a currently listed (FO, 67) Eritreo-Arabian/East African species.

New combinations

Inula stenocalathia (RECH F.) SOLDANO, comb. nov. = *Codonocephalum stenocalathium* RECH. F., Fl. Iranica 145: 74 (1980).

Ref.: FO, 8: 30.

The inclusion of *Codonocephalum* FENZL in *Inula* L. is supported by ANDERBERG's (1991; see also ANDERBERG 1994) accurate study on Inuleae, where other "*Codonocephalum*" of the same country (*inuloides* and *peacockianum*) are listed under *Inula*. *I. stenocalathia* is an Iranian endemite.

Seriphidium densiflorum (VIV.) SOLDANO, comb. nov. = *Artemisia densiflora* VIV., Fl. Cors. App. Alt.2, 4 t. 2 (1830).

Ref.: FI, 3: 108.

This species is endemic to Northern Sardinia/extreme South Corsica. The specific rank adopted in FI, by CORRIAS (1986) - with accurate taxonomy and typification - and by GEHU et al. (1989) is lowered to the subspecific one in FF.

Stemmacantha caulescens (COSSON et BALANSA) SOLDANO, comb. nov. = *Rhaponticum caulescens* COSSON et BALANSA, Bull. Soc. Bot. France 20: 251 (1873). = *Leuzea caulescens* (COSSON et BALANSA) HOLUB, Folia Geobot. Phytotax. 8(4): 391 (1973).

Ref.: JAHANDIEZ & MAIRE 1934: 820.

Stemmacantha imatongensis (PHILIPSON) SOLDANO, comb. nov. = *Centaurea imatongensis* PHILIPSON, Jour. Bot. (London) 77: 232 (1939). = *Rhaponticum imatongense*

(Philipson) Sojàk, Novit. Hort. Bot. Univ. Carol. Prag. 1962: 48 (1962). = *Leuzea imatongensis* (Philipson) Holub, Folia Geobot. Phytotax. 8(4): 391 (1973).

Ref.: Lisowski 1991: 594.

Rhaponticum, as indicated in the references of these two species, is a controversial abandoned name (cfr. Holub 1973, Dittrich 1984) and *Stemmacantha* Cass. is the one currently listed (Bremer 1994; FO; FR etc.).

Tanacetum musili (Velen.) Soldano, comb. nova = *Pyrethrum musili* Velen., Sitz.-Ber. Boehm. Ges. Wiss. 1911, 11:11 (1912).

Ref.: FO, 8: 60.

Pyrethrum Zinn is currently reduced to *Tanacetum* L. (cfr. Bremer & Humphries 1993). The species in argument is a Saharo-Arabian endemite.

Wedelia ciliata (Schum.) Soldano, comb. nov. = *Verbesina ciliata* Schum. in Schum. & Thonn., Beskr. Guin. Pl.: 391 (1827). = *Aspilia ciliata* (Schum.) Wild, Kirkia 6: 41 (1967).

Ref.: FO, 8: 39.

Wedelia kotschyi (Sch.Bip. ex Hochst.) Soldano, comb. nov. = *Dipterotheca kotschyi* Sch.Bip. ex Hochst., Flora (Intell.) 25: 435 (1842). = *Aspilia kotschyi* (Sch.Bip. ex Hochst.) Benth. et Hook. ex Oliv., Trans. Linn. Soc. London 29: 98 (1875).

Ref.: FO, 8:39.

The last two combinations for these species of Tropical Africa and Eritreo-Arabian province is a consequence of the reduction of *Aspilia* to *Wedelia*.

Neglected priorities

Anthemis saxatilis Lam. et DC., Syn. Pl. Fl. Gall.: 291 (1806), ante DC. ex Willd., Enum. Pl. Horti Berol.: 910 (1809).

Ref.: FF, 16.

Crepis lyrata (Ledeb.) Turcz., Bull. Soc. Nat. Moscou 11: 96 (1838), ante (Ledeb.) Froel. in DC., Prodr. 7: 170 (1838).

Ref.: FR, 55.

Turczaninow's paper listing this Siberian endemite antedates de Candolle's name published in the same year (cf. Stafleu & Cowan 1985).

Crepis suffreniana (DC.) STEUDEL, Nomencl. Bot., ed. 1: 236 (1821), ante LLOYD, Fl. Loire-Inf.: 155 (1844).

Ref. FE, 5:356; FI, 3: 280; FF, 53.

Leucanthemum halleri (VITMAN) DUCOMMUN, Taschenb. Schw. Bot.: 383 (1869). = *Chrysanthemum halleri* VITMAN, Summa Pl. 5: (1791), ante SUTER, Fl. Helv. 2: 193 (1802).

Ref.: HESS et al. 1972: 574.

VITMAN's description of this endemite of Switzerland and Austria Alps exactly reproduces SUTER's one and both are based on HALLER (1768 n.97). DUCOMMUN's combination based on SUTER's name is a bibliographic citation error that does not invalidate it (art 33.3 of the Code).

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Nature of ergastic substances in some Asteraceae seeds - VII

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Abstract

Seeds of 136 species of Asteraceae distributed in 68 genera were examined for alkaloids, fats and oils, inulin, protein, starch granules and tannin. All the examined species are herbs. Fats and oils, inulin and protein were found to be present in all the investigated taxa and alkaloids were absent in 48 species. 10 taxa indicated positive for tannin, and starch grains were found to be absent in all the investigated species except two (*Cyathula prostrata* and *Vernonia macrocyanus*).

Introduction

Seeds apart from being the chief source of propagation of plants are also principal storage organ of ergastic substances. A study of the distribution of the stored food products (often end products of metabolism) shows positive correlation with other morphological characteristics and has proved to be of diagnostic taxonomic value. According to Gill & Ayodele (1986), the amount of cultivated crops is relatively in sufficient to provide for the world food supply and hence the knowledge of stored products in the seeds of wild plants cannot be over-emphasized and this can be done with a view to harness the resources of the wild plants. Also the future energy needs of man will rely heavily on renewable plant resources to replace the presently decreasing fossil fuel reserve (Abelson 1998).

Calvin (1983) observed that relatively few plants have been identified as potential sources of fuel.

The importance of the nature of ergastic substances in plant systematics has been stressed by various workers, e.g. Tatteoka (1955, 1962) Earle & Jones (1962), Maheshwari & Chakrabarty (1967), Smith (1976), Gill et al. (1980, 1984, 1991), Omoigui & Gill (1988), and Gill & Abili (1989).

The present paper reports on the investigations of ergastic substances in 136 taxa of Asteraceae from 68 genera.

Materials and methods

Seeds of 136 taxa from 68 genera were obtained from Botanischer Garten und Botanisches Museum, Berlin-Dahlem, Germany. Vouchers of the seeds examined are kept in the Botany Department of the University of Benin, Benin City, Nigeria. Chemical tests of various ergastic substances were carried out following the procedures described by Gill et al. (1991).

Results

The results of taxa studied for their ergastic substances along with their habit are summarized in Table 1. The taxonomic arrangement of the taxa under this family is alphabetical.

Discussion

For more than three decades now, much attention has been focused on the comparative studies of basic molecules in relation to taxonomic problems. Bate-Smith & Lerner (1954) were probably the first to study the leuco-anthocyanins in flowering plants and concluded that ligneous taxa showed positive tests for them whereas they are of re-occurrence in herbaceous taca. De Wet & Scott (1965) are of the opinion that essential oil can be used as a taxonomic criterion and according to them, chemical characters are often found to be more reliable that gross morphology in determining taxonomic affinities. According to Erdtman (1956), these ergastic substances are secondary products of plant metabolism which must have been formed in certain metabolic processes and are retained when the taxon in question undergoes further evolution. Knowledge of the principle and direction of chemical processes might contribute to an understanding of the phylogenetic relations of present day plant taxa.

All taxa studied indicated the presence of fats and oils, inulin and proteins. 48 taxa were devoid of alkaloids and 10 taxa indicated positive for tannin, while 2 taxa, viz. *Cyathula prostrata* and *Vernonia macrocyanus* indicated for starch. The presence of starch in these two species shows their primitive character over those species that do not have starch.

Earle & Jones (1962), Gill & Ayodele (1986) and Omoigui & Gill (1988) earlier reported the presence of various ergastic substances in 11 plant families. They did not report any incidence of starch. However, forms of ergastic substance were the same as in the present report.

Gill et al. (1980, 1984, 1991) have established a relationship between life forms and the nature of ergastic substances. The observed that starch grains are often associated with a herbaceous habit, however from the present study, it is obvious that no such correlation exists in the family Asteraceae as only two investigated taxa showed the presence of starch.

Taxa with the presence of fats and oils should be further investigated qualitatively to determine the nature of fats and oils and suitability for commercial exploitation.

Acknowledgements

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Table 1. Nature of ergastic substances in Asteraceae taxa studied.

Taxon	Life form*	Alkaloid	Fats and oil	Inulin	Protein	Starch	Tannin
1	2	3	4	5	6	7	8
Achillea abrotanoides (Vis.) Vis.	H	+	+	+	+	-	-
Achillea ageratum L.	H	+	+	+	+	-	-
Achillea coarctata Poir.	H	+	+	+	+	-	-
Achillea crithmifolia Waldst. & Kit.	H	+	+	+	+	-	-
Achillea grandifolia Frivald	H	+	+	+	+	-	-
[1]*Achillea millefolium* L.	H	+	+	+	+	-	-
Achillea ptarmica L.	H	+	+	+	+	-	-
Achillea teretifolia Willd.	H	+	+	+	+	-	-
[3]*Ageratum conyoides* L.	H	-	+	+	+	-	-
Ageratum houstonianum Mill.	H	-	+	+	+	-	-
Anaphalis triplinervis (Sims) C. B. Clarke	H	-	+	+	+	-	-
Antennaria alpina (L.) Gaertn.	H	+	+	+	+	-	-
Anthemis macedonica Boiss. & Orph.	H	+	+	+	+	-	-
Anthemis tinctoria L.	H	+	+	+	+	-	-
Anthemis tinctoria L. subsp. *australis* R. R. Fernandes	H	+	+	+	+	-	-
Anthemis tinctoria var. *pallida* DC.	H	+	+	+	+	-	-
Anthemis triumfettii (L.) All.	H	+	+	+	+	-	-
[1]*Arctium lappa* L.	H	+	+	+	+	-	-
[2]*Arctium minus* Bernh.	H	+	+	+	+	-	-
Arctium nemorosum Lej.	H	+	+	+	+	-	-
Arctotis venusta Norlindh	H	+	+	+	+	-	-
Artemisia pedemontana Balbis ex Lois.	H	+	+	+	+	-	-
Aster alpinus L.	H	+	+	+	+	-	-
Aster amellus Bess. subsp. *ibericus* (Stev.) Avetisyan	H	+	+	+	+	-	-
Aster cordifolius L.	H	+	+	+	+	-	-
Aster foliaceus Lindl. var. *cusickii* (A. Gray) Cronq.	H	+	+	+	+	-	-
Aster pyrenaeus DC.	H	+	+	+	+	-	-
Aster tripolium L.	H	+	+	+	+	-	-
Asteriscus sericeus DC.	H	-	+	+	+	-	-
Bidens cernua L.	H	+	+	+	+	-	-
Bidens leucantha (L.) Willd.	H	+	+	+	+	-	-
Bidens pilosa L.	H	+	+	+	+	-	-
Carduus crispus L.	H	+	+	+	+	-	-
Carduus nutans L.	H	+	+	+	+	-	-

1	2	3	4	5	6	7	8
Carduus tmoleus Boiss. subsp. *armatus* (Boiss. & Heldr.) Franco	H	+	+	+	+	-	-
Carlina vulgaris L.	H	+	+	+	+	-	-
Catananche caerulea L.	H	-	+	+	+	-	-
Centurea jacea L.	H	-	+	+	+	-	-
Centaurea ornata Willd.	H	-	+	+	+	-	-
Centaurea salonitana Vis.	H	+	+	+	+	-	-
Chamomilla suaveolens (Pursh.) Rydb.	H	+	+	+	+	-	-
Chaptalia arechavaletai Arechav.	H	+	+	+	+	-	-
Chondrilla juncea L.	H	+	+	+	+	-	-
Chromolaena odorata (L.) R.M. King & Robinson	H	-	+	+	+	-	-
Chrysanthemum coronarium L.	H	-	+	+	+	-	-
Chrysanthemum segetum L.	H	-	+	+	+	-	-
Chrysopsis villosa (Pursh.) Nutt.	H	-	+	+	+	-	-
[1]*Cirsium arvense* (L.) Scop.	H	+	+	+	+	-	-
Cirsium candelabrum Griseb.	H	+	+	+	+	-	-
Cirsium flavispina DC.	H	-	+	+	+	-	-
Cirsium monspessulanum (L.) Hill.	H	+	+	+	+	-	-
Cirsium palustre (L.) Scop.	H	+	+	+	+	-	-
Cirsium trachylepis Boiss.	H	+	+	+	+	-	-
[1]*Cirsium vulgare* (Savi) Ten.	H	-	+	+	+	-	-
Conyza bonariensis (L.) Cronq.	H	-	+	+	+	-	-
Conyza canadensis (L.) Cronq.	H	+	+	+	+	-	-
Coreopsis lanceolata L.	H	+	+	+	+	-	-
Crassocephalum crepidioides (Benth.) S. Moore	H	+	+	+	+	-	-
Crepis commutata (Spreng.) Babc.	H	+	+	+	+	-	-
Crepis foetida L. subsp. *foetida*	H	+	-	+	+	-	-
Crepis multiflora Sm.	H	+	+	+	+	-	-
Crepis pyrenaica (L.) Greuter	H	+	+	+	+	-	-
Crepis rhoeadifolia (M. Bieb.) Celak.	H	+	+	+	+	-	-
Cyathula prostrata (L.) Blume	H	-	+	+	+	-	-
Dendroseris micrantha Hook. & Arn.	H	+	+	+	+	-	-
Dimorphotheca pluvialis (L.) Moench	H	-	+	+	+	-	-
Dittrichia graveolens (L.) Greuter	H	+	+	+	+	-	-
Doronicum austriacum Jacq.	H	-	+	+	+	-	-
Dyssodia setifolia (Lagasca) Robinson var. *radiata* (Gray) Strother	H	+	+	+	+	-	-
Echinops albidus Boiss. & Sprun.	H	-	+	+	+	-	-

1	2	3	4	5	6	7	8
Emilia sonchifolia L.	H	+	+	+	+	-	-
Erigeron speciosus (LINDL.) DC.	H	+	+	+	+	-	-
[1]*Eupatorium cannabinum* L.	H	+	+	+	+	-	-
Eupatorium purpureum L.	H	-	+	+	+	-	-
Gaillardia aristata PURSH.	H	-	+	+	+	-	-
Galinsoga ciliata (RAF.) S. F. BLAKE	H	+	+	+	+	-	-
Galinsoga parviflora CAV.	H	+	+	+	+	-	-
Gerbera anandria (L.) SCH. BIP.	H	+	+	+	+	-	-
Gnaphalium sylvatium L.	H	-	+	+	+	-	-
Helianthus annuus L.	H	-	+	+	+	-	-
Helichrysum rupestre (RAF.) DC.	H	-	+	+	+	-	-
Hieracium amplexicaule L.	H	-	+	+	+	-	-
Hieracium bornmuelleri FREYN.	H	-	+	+	+	-	-
Hieracium sabaudum L.	H	-	+	+	+	-	-
Hyochoeris maculata L.	H	-	+	+	+	-	-
Hyochoeris oligocephala (SVENT. & BRAMW.) LACK	H	+	+	+	+	-	-
Hypochoeris radicata L.	H	+	+	+	+	-	-
Hypochoeris unifora VILL.	H	-	+	+	+	-	-
Inula orientalis LAM.	H	+	+	+	+	-	-
Inula salicina L.	H	+	+	+	+	-	-
Inula thapsoides (WILLD.) SPR.	H	-	+	+	+	-	-
Inula verbascifolia (WILLD.) HAUSSKN. subsp. *aschersoniana* (JANKA) TUTIN	H	+	+	+	+	-	-
Jurinea alata (DESF.) CASS.	H	+	+	+	+	-	-
[1]*Lactuca serriola* L.	H	-	+	+	+	-	-
[1]*Lapsana communis* L.	H	-	+	+	+	-	-
Leontodon autumnalis L.	H	-	+	+	+	-	-
Leontodon glabratus (W. KOCH) BISCH.	H	+	+	+	+	-	-
Leontodon hispidus L.	H	-	+	+	+	-	-
Leuzea centauroides (L.) HOLUB	H	-	+	+	+	-	-
Matricaria maritima L.	H	+	+	+	+	-	-
Onopordum acanthium L.	H	+	+	+	+	-	-
Onopordum bracteatum BOISS. & HELDR. subsp. *ilex* (JANKA) FRANCO	H	-	+	+	+	-	-
Onopordum illyricum L.	H	+	+	+	+	-	-
Picris evae LACK	H	-	+	+	+	-	-
Porophyllum ruderale L.	H	-	+	+	+	-	-
Pulicaria dysenterica (L.) BERNH.	H	+	+	+	+	-	-
Pulicaria odora (L.) REICHENB.	H	+	+	+	+	-	-
Santolina chamaecyparissus L.	H	+	+	+	+	-	+
Santolina rosmarinifolia L.	H	+	+	+	+	-	-

1	2	3	4	5	6	7	8
Santolina squarrosa (DC.) NYMAN	H	-	+	+	+	-	+
Sanvitalia procumbens LAM.	H	+	+	+	+	-	-
Scorzonera cretica WILLD.	H	+	+	+	+	-	-
Senecio adonidifolius LOIS.	H	-	+	+	+	-	-
Senecio appendiculatus (L.F.) SCH. BIP.	H	+	+	+	+	-	-
Senecio aquaticus HILL.	H	+	+	+	+	-	-
Senecio chrysanthemoides DC.	H	+	+	+	+	-	-
Senecio doria L.	H	+	+	+	+	-	-
Senecio gnaphalodes SIEB. var. *gnaphalodes*	H	+	+	+	+	-	-
Senecio hansenii KUNKEL	H	-	+	+	+	-	-
Senecio thapsoides DC. subsp. *thapsoides*	H	+	+	+	+	-	-
Sonchus arvensis L.	H	-	+	+	+	-	-
Spilanthes ocymifolia (LAM.) A. H. MOORE	H	-	+	+	+	-	-
Staehelina uniflosculosa (SIBTH. & SM.)	H	+	+	+	+	-	-
Tanacetum cilicium (BOISS.) GRIERSON	H	+	+	+	+	-	-
Tanacetum parthenium (L.) SCH. BIP.	H	+	+	+	+	-	-
Tanacetum pseudoachillea C. WINKL.	H	+	+	+	+	-	-
Tanacetum vulgare L.	H	+	+	+	+	-	-
Telekia speciosa (SCHREB.) BAUMG.	H	-	+	+	+	-	-
Telekia speciosissima (L.) LESS.	H	+	+	+	+	-	-
Tragopogon tommasinii SCH. BIP.	H	+	+	+	+	-	-
[3]*Tridax procumbens* L.	H	+	+	+	+	-	-
Vernonia macrocyanus O. HOFFM.	H	-	+	+	+	-	-
Xanthium spinosum L.	H	+	+	+	+	-	-
Xanthium strumarium L. subsp. *italicum* (MORETTI) D. LOVE	H	-	+	+	+	-	-
Zinnia peruviana (L.) L.	H	+	+	+	+	-	-

* = Herb L = Liana S = Shrub T= Tree

[1]- Species earlier investigated by GILL & ABILI (1989)
[2]- Species earlier investigated by EARLE & JONES (1962)
[3]- Species earlier investigated by OMOIGUI & GILL (1988)

Allelopathic effect of *Calotropis procera* on the germination of *Helianthus annuus* seeds

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Abstract

The effect of 2 %, 10 % w/v aqueous extract of *Calotropis procera* leaves and flowers on the germination of nine (9) cultivars of *Helianthus annuus* seed was investigated.

The interaction resulted in poor germination performance of below 30 % in all the cultivars, while the control (distilled water) treatment recorded high performance of above 60 % germination. Only a slight difference in inhibitory effect was recorded between the different extract concentrations and plant parts solution extract treatments.

Introduction

From De Candolle (1832) to date, a lot of work has been carried out on the allelopathic effect of plants, both agricultural or non-agricultural, on the growth performance of other plants. Such plants are believed to affect their neighbour's performance by direct releases of the allelochemicals (Rice 1984, Garcia & Anderson 1984, Gill et al. (1993), or indirectly through the effect of the toxins on the characteristics of the growth media (soils) where they both survive, by altering the physical as well as chemical characteristics of the soil (Igboanugu 1986, Inderjit & Dakshini 1994).

Calotropis procera is a common weed of most farm lands in the northern part of Nigeria. It has been speculated that it affects the performance of most crop plants in fields where it exists, possibly due to its high alkaloid and glucoside content (Bouguent 1972, Daubenmire 1974, Gbile 1986). However, this remains only a supposition as no investigation of such claim has been undertaken with respect to *Helianthus annuus.*

Thus, the aim of the present study is to ascertain the inhibitory effect of *Calotropis procera* plant (parts) leachates on the germination of *Helianthus annuus* seeds.

Materials and methods

Helianthus annuus seeds (9 cultivars: V_1 - Funtua, V_2 - Cakinki, V_3 - Saturn, V_4- Record, V_5 - Perodirk, V_6 - Cherniank, V_7 - Isa-anka, V_8 - Vnumik and V_9 - Smena), used for this study were collected from AFCOTT (AFCOTT Nig. Ltd, Ngurore South-East Yola, Nigeria) through a seed exchange programme.

Leaf and flower components of *Calotropis procera* plants were collected in August 1995 from Yola, Nigera. The fresh materials were subdried and ground. 2g and 10g weight of each of the leaf and flower parts were dissolved in 100 mls of distilled water to give 2 % weight/volume (w/v) and 10 % w/v extract solutions of the leaf/ flower components of the *C. procera* plant. The preparations were filtered and refrigerated. 150 seeds of each of the 9 *Helianthus annuus* cultivars were divided into 5 parts of 30 seeds for each treatment (2 %, 10 % leaves; 2 %, 10 % flower and control). Each set of 30 seeds were further subdivided into triplicates of 10 seeds. Petri-dishes were lined with filter paper each with 10 *Helianthus annuus* seeds, and moistened daily with the 2 %, 10 % leaves and flowers solution of *C. procera*. The control treatment was moistened with distilled water. The experimental set up was kept at constant temperature ($30^{\circ} \pm 3^{\circ}$ C) in a growth chamber. Germination was recorded regularly at 48 hrs interval.

Results

Table 1 shows the germination percentages recorded for the nine (9) cultivars of *Helianthus annuus*. The results obtained show a marked response of germination inhibition in all the cultivars used. The control treatment recorded a high performance for all the cultivars, with the lowest percentage germination of 62 %, and the highest of 78 % in the cultivars Funtua (V_1) and Vnumik (V_8) respectively. The maximum recorded for any of the extract solutions treatment was 21 % for the cv. Cherniank (V_6) with the cv. Funtua (V_1), Cakinki (V_2) and Record (V_4) recording the minimum germination of 10 %. The variance level using Duncan's multiple range test ($P = 0.05$) further shows the significance level between the control (distilled water) treatment and the extract solutions treatments. However, only slight significance difference existed between the different concentrations (2 %, 10 %) of the leaves or flowers solutions and between the different plant component (parts) - leaves and flowers solution treatments (Table 1).

Discussion

The phenomenon of allelopathy is not only an important one, but also plays a significant role in the distribution of plants in fields. From the present study, it is quite apparent that *Calotropis procera* extract solutions greatly hindered the germination of *H. annuus* seeds. This suppressive effect may be due to the presence of noxious compounds in the species *C. procera* and the genus as a whole. The incidence of such toxins was first elucidated by WATT & BREYER-BRANDWIJK (1962). They showed that species of *Calotropis* contained a strong cardiac poison in the latex exudate known as "Calotropin". BOUGUENT (1972) and DAUBENMIRE (1974) identified 7 glucosides as well as calotropin in the latex of some members of the genus *Calotropis*. Later, GBILE (1986) extracted the following alkaloids; Benzoythcolone, Benzylusolineolone, Calotropin, Calotoxin, Uscharin, Uscharuchin, Calactin vorisherine and mudarin from this group of plants.

The presence of an extract solution of a whole plant or plant parts with an array of toxic compounds such as above in the growth medium of another plant, can be directly linked to the inhibition of growth of the effector plant (EVANARI 1949, RICE 1984), or indirectly by altering the characteristics (physical and chemical) of the growth media (soils), and/or the availability of nutrient, pH, total phenolic levels (TPL), and microbial population (BLUM & SHAFER 1988, INDERJIT & DAKSHINI 1992, 1994).

The toxicity of the bioassay 2 % or 10 % w/v plant part was undoubtedly pronounced. Table 1 shows the percentage germination recorded for the five different treatments on the 9 cultivars of *H. annuus*. The control treatment (distilled water) recorded high germination of above 60 % for all the cultivars, with highest of 78 % for the cv. Vnumik (V_8) and the lowest of 62 % for the cv. Isa-anka (V_7), whereas, the highest recorded for any of the 4 extract solutions was 21 % in the 2 % w/v leaf extract solution treatment for the cv. Cherniank (V_6). Similarly, ADAMS & AZIMI (1991) showed the suppressive effect of *Cyperus rotundus* leaf extract on the germination of wheat grains. Also, GILL et al. (1993) reported on the allelopathic effect of *Chromolaena odorata* extract on growth of cowpea.

A high degree of variance in treatment effect between the control and extract solutions was recorded. This further supports the view that the toxins present in the effector plant's extract solutions inhibited germination growth in seeds of *H. annuus*.

A significant level of treatment effect amongst the leaf and flower extract solutions and at different concentrations (2 % & 10 % w/v) was not recorded, as only the cv. Funtua (V_1) showed some level of significance of treatment effect for the leaf extract treatments, while the cv. Funtua, Cakinki and Record recorded identical results for the flower extract treatments.

Similar results from OGBE et al. (1994), showed that the leaf extract of *Chromolaena odorata* inhibited germination seedling growth in *Zea mays* grain to a high degree irrespective of the duration of extraction. Unlike in most allelopathic studies, the different plant component (parts) extract solutions, in the present study, show no significant level of difference in their effect on the germination of seed of *H. annuus.* This is because identical degrees of inhibition were exhibited by both leaf and flower extract solutions.

In conclusion, the authors are of the view that regardless of the component part extracted, the concentration of the extract solution of *C. procera* or the cultivar of *H. annuus* seeds employed, the degree of inhibition is similar. A comparable degree of the allelopathic effect of *C. procera* on other plants is apparent from the present study. This suppressive ability might account for the spatial distribution and reduced growth of plants and notably *H. annuus* in fields where it occurs.

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Table 1. Allelopathic effect of *Calotropis procera* leaves and flowers extract solutions on germination of *Helianthus annuus* seeds.

Treatment	Cultivars								
	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
Control	60a	70a	70a	66a	64a	64a	62a	78a	64a
Leaves 2 % w/v	18b	17b	20b	17b	16b	21b	13b	18b	15b
Leaves 10 % w/v	10d	13c	18b	15b	12c	16c	12c	19b	15b
Flower 2 % w/v	14c	17b	20b	18b	13b	15c	16b	16b	12b
Flower 10 % w/v	10d	10c	17b	10c	13b	11b	14b	12c	13b

* Within each variable (cultivars), means followed by the same subscript are not significantly different at 5 % level.

** w/w = weight/volume

Key to names of cultivars:

V_1 — Funtua
V_2 — Cakinki
V_3 — Saturn
V_4 — Record
V_5 — Perodirk
V_6 — Cherniank
V_7 — Isa-anka
V_8 — Vnumik
V_9 — Smena

Germination control of *Helianthus annuus* L. using two growth regulators - thiourea and coumarin

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Abstract

Thiourea effectively promoted germination in 9 varieties of *Helianthus annuus* seeds. Conversely, coumarin retarded germination growth in the seeds. A significant level of difference was observed (at 5 % level) between the treatments, with thiourea recording as high as 85 %, 70 % germination under light and dark condition, and a minimum degree of 43 % and 38 % germination in the cultivars, "Funtua" (V_1) and "Vnumik" (V_8), respectively. Coumarin recorded low percentage germination (39 %, 27 %) in the cv. "Saturn" (V_3) with the lowest percentage germination of 10 % in cv. "Record" under the light condition. The cultivars "Funtua" (V_1) and "Cakinki" (V_2) had better germination energies than the others, with cv. "Vnumik" (V_8) exhibiting the least. The light condition enhanced germination in *H. annuus*.

Introduction

Seeds have been shown to differ in their response to growth regulatory compounds; this applies to both hormonal and non-hormonal compounds. Likewise, these growth chemicals whether applied exogenously or naturally occurring exert either stimulatory or inhibitory effects under different conditions and concentrations, thus either as germination stimulators, or germination inhibitors when they suppress or nullify germination growth (Mayer & Poljakoff-Mayber 1989).

Growth regulators (stimulators and inhibitors) commonly occur naturally and interact in seeds to effect dormany/germination together with other dormancy/germination mechanisms present in the seed or its environment (Kelly et al. 1992). They can be synthesized and exogenously applied to achieve the same effect as the endogenously present ones. Most often these interactive regulators act antagonistically; a commonly

known interactive pair is the GA/ABA Complex (KETRING 1977, EGLEY 1972) which occurs in growth regions of plant components. Similarly, the pair thiourea and coumarin have been reported for seeds, where they control the germination growth of such seeds. In the case of lettuce seeds, while thiourea effectively stimulated germination to as high as 100 %, coumarin reduced germination from 50 % level (control) to zero. Such interactions are light, temperature and respiration linked (MAYER & POLJAKOFF-MAYBER 1989).

The present study sets to determine the effect of exogenously applied thiourea and coumarin on the germination growth of *Helianthus annuus* seeds.

Materials and methods

Seeds for the study were obtained from AFCOTT (AFCOTT Nigeria Plc, Ngurore, 25km South-East Yola, Nigeria). Seeds of 9 cultivars: V_1 - Funtua, V_2 - Cakinki, V_3 - Saturn, V_4 - Record, V_5 - Perodirk, V_6 - Cherniank, V_7 - Isa-anka, V_8 - Vnumik and V_9 - Smena, whose healthy state have been determined to be 100 % with tetrazolium salt, were used. 5g/L thiourea solution and 0.04g/100ml coumarin solution were employed for the experiment.

The three treatments, viz. control (A), thiourea (B) and coumarin (C) were set up for light and dark condition treatments (A1 and A2, B1 and B2, C1 and C2). Each illumination regime treatment was replicated in triplicates for each cultivar. Following a randomized design 180 seeds of each cultivar were selected for the triplicated treatments under the light and dark conditions. Treated seeds were placed in filter-paper lined petri-dishes. The A1, B1, C1 sets were placed under continuous light, while the A2, B2, C2 were placed under continuous dark condition. Germination was recorded at 2 day intervals for 28 days.

Statistical analysis was carried out using Duncan's multiple range test at 5 % level ($P = 0.05$).

Results and discussion

Figs. 1 and 2 show the percentage germination of treatments for the 9 cultivars of *H. annuus* under light and dark conditions. Thiourea recorded the highest germination of 85 % under the light condition for the V_1, this is higher than that recorded under the dark condition (70 %) for the same cultivar. The smallest percentage recorded for the thiourea treatment was 43 % and 38 % under both illumination regimes respectively for the cv. V_8 (Vnumik). The control treatment recorded a maximum germination of

70 % for V_1 (Fig. 1, Table 1) and the minimum levels were above 30 %. However, germination percentages recorded for the coumarin treatment were low, with the highest of above 53 % and the lowest 10 % for the same cultivar, V_4 (Figs. 1 and 2).

From the foregoing, it is apparent that thiourea irrespective of the illuminaton conditions or kinds of cultivars, stimulated germination to a high level, while coumarin exhibited inhibitory effect. These stimulatory/inhibitory effects of thiourea and coumarin may have been due to the action on the storage materials of the seeds, the oxidative phosphorylation (phosphate/oxygen ratio) and the coupling action in germinating seeds either directly or indirectly; this view is supported by MAYER & POLJAKOFF-MAYBER (1989). Germination was higher for all the cultivars for control, thiourea and coumarin treatment under the light condition, suggesting light dependency (stimulatory effect) of the seeds of *H. annuus*. HSIAO et al. (1988) showed the stimulatory effect of growth stimulators on germination of the witchweed (*Striga asiatica*). BASKIN & BASKIN (1974) also reported up to 100 % germination in seeds of *Isanthus brachiatus* treated with gibberellic acid. UGBOROGHO & AGOMO (1989) showed the germination retarding effect of colchicine on seeds of *Vigna unguiculata*. The germination enhancing effect of light was demonstrated by BASKIN & BASKIN (1975) in *Helenium amarum* seeds.

Analysis of variance (Table 1) showed a marked and significant level of difference between thiourea and coumarin treatments. Significant differences were recorded in some of the cultivars between the control and thiourea treatments. Also a major level of difference was apparent between the control and coumarin treatments. Within the same treatment, significant difference was recorded between the light and dark regimes with the light treatment recording a higher degree of germination than the dark in all cases. From the comparison, the thiourea treatment showed a higher degree of germination in all the cultivars, and the coumarin treatment recorded the lowest degree. Also, the cultivars V_1 and V_2 recorded higher level of germination, and cv. V_8 obtained the lowest germination for all the treatments.

From the above it is quite conclusive that thiourea stimulates germination while coumarin retards germination in *H. annuus* seeds. The cv. Funtua and Cakinki are the most readily germinating cultivars while cv. Vnumik shows the lowest initial germination energy.

The stimulatory effect of light on germination of *H. annuus* seeds is an area of further investigation.

The performance of the cultivars in the present study can be useful in the delimitation of infraspecific taxa in a complex species such as *Helianthus annuus*.

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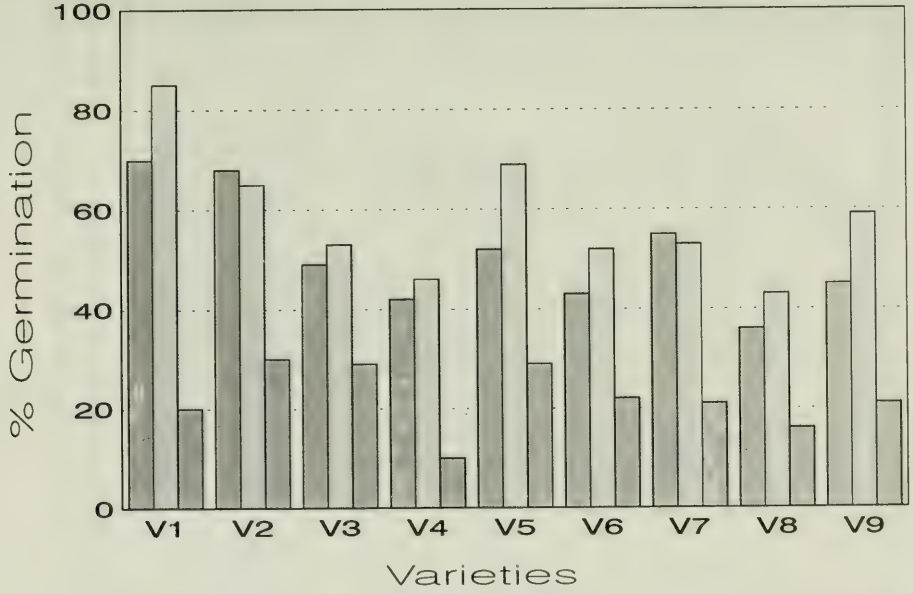
Table 1. Germination percentages (P = 0.05) of *Helianthus annuus* seeds treated with thiourea and coumarin and of untreated (control) seeds under light and dark condition.

Cultivar	Treatments					
	Control		Thiourea		Coumarin	
	light	dark	light	dark	light	dark
V_1	70b	63b	85a	70b	20c	20c
V_2	68a	57b	65a	62a	30c	25c
V_3	49a	43b	53a	52a	39b	27c
V_4	42b	40b	46a	38b	10d	33c
V_5	52b	43c	69a	53b	29d	23d
V_6	43b	41b	52a	52a	22c	18c
V_7	55a	34b	53a	52a	21c	19c
V_8	36a	32b	43a	38a	16c	18c
V_9	45b	40b	59a	43b	21c	14c

Percentages followed by the same letter are not significantly different at 5 % level.

Key to cultivars:

V_1 — Funtua
V_2 — Cakinki
V_3 — Saturn
V_4 — Record
V_5 — Perodirk
V_6 — Cherniank
V_7 — Isa-anka
V_8 — Vnumik
V_9 — Smena



V_1 — Funtua
V_2 — Cakinki
V_3 — Saturn
V_4 — Record
V_5 — Perodirk
V_6 — Cherniank
V_7 — Isa-anka
V_8 — Vnumik
V_9 — Smena

Fig. 1. Effect of hormones on seed germination of H.annuus under light condition

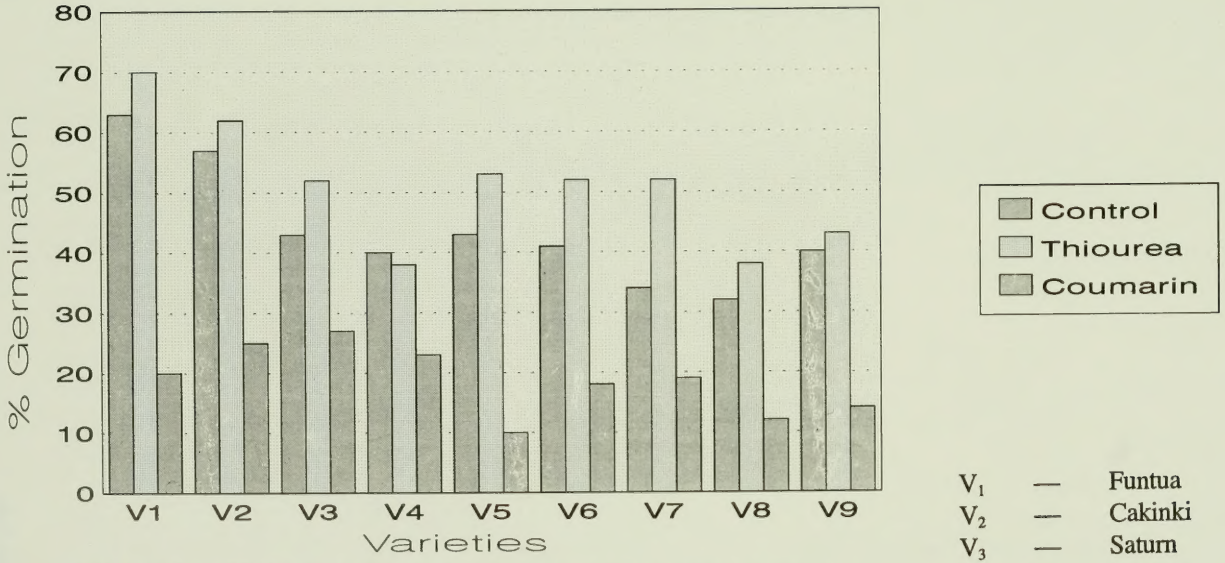


Fig. 2. Effect of hormones on seed germination of H. annuus under dark condition

V_1 — Funtua
V_2 — Cakinki
V_3 — Saturn
V_4 — Record
V_5 — Perodirk
V_6 — Cherniank
V_7 — Isa-anka
V_8 — Vnumik
V_9 — Smena

Elanders **gotab**, Stockholm 1998