American Journal of Plant Sciences, 2014, 5, 1202-1211
Published Online April 2014 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2014.59133

Situation of Biofungicides Reconnaissance, a Case of Anthracnose Disease of Cowpea

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Received 8 January 2014; revised 6 March 2014; accepted 21 March 2014

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Abstract

Plant extracts have long been used in commercial agriculture as anti-microbial tools in food safety applications. These offer growers and agrobiologists many unique benefits which include their eco-friendliness. This work reviews the situation of Biofungicides reconnaissance in reference to fungal disease of cowpea. Twenty different pathogens were associated with various fungal diseases of cowpea and, only the species of *Colletotrichum* was found to have the virulence and propensity of afflicting a 100% infection on a single susceptible cowpea crop. Plant families under the affliction of *Colletotrichum* were analyzed. The different forms of botanicals so far availed for use as potential biofungicidal were identified. Eighteen plant families were found to represent the entire plants and plant materials agrobiologically screened within a range of thirteen years and found to habour large spectra of species containing substances of biofungicidal potentials. Current position in the use of Botanicals to combat agricultural pests and disease is 7% of the total cowpea disease management options.

Keywords

Anthracnose; Biofungicides; Biopesticides; *Colletotrichum destructivum*; Cowpea.

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is a leguminous, annual grain crop in the bean family (Fabaceae/Leguminosae), with high degree of variation in growth habit, leaf shape, flower colour and seed size and colour, cultivated mostly in the humid tropics of the globe for its seeds, as a vegetable crop, green manure, fodder, as a cash crop and or cover crop [1]-[4]. The crop's haulms are also valuable source of livestock protein [5].

Cowpea is a native to Africa where they are often intercropped with maize (*Zea mays* L.), Sorghum (*Sorghum*

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How to cite this paper: Obi, V.I. and Barriuso-Vargas, J.J. (2014) Situation of Biofungicides Reconnaissance, a Case of Anthracnose Disease of Cowpea. *American Journal of Plant Sciences*, **5**, 1202-1211. http://dx.doi.org/10.4236/ajps.2014.59133

bicolor (L.) Moench), Pearl millet (*Pennisetum americanum* (L.) Leeke) and Cassava (*Manihot esculenta* Crantz) [6] [7]. This all important global crop, encounters a number of operational constraints, including pests and diseases that limit its production and yield potentials from seedling to harvest [3] [4] [7] and often provoking grain yield loss of over 35% [8] [9].

Major pathogenic groups associated with cowpea diseases, include: fungi, bacteria, viruses, nematodes and the parasitic flowering plants [3]. And Anthracnose is its major disease, causing severe damage and loses under low temperature, high humidity and free moisture [3] [6]. Though the major fungal pathogen of Cowpea crop is the *Colletotrichum destructivum* O'Gara, there are some other old and new fungal pathogens that influence its existence, reproduction and survival. Some of these pathogens attack and infect the roots of the crop [10]; stems [11] [12]; leaves [3] [13] [14]; pods and fruits [15] [16]; seeds/seedlings [17] [18]; whole plant parts [4].

C. destructivum is polycyclic, having multiple life cycles in a growing season [19], and hemibiotrophic, thriving in both living and dead tissues [4]. The pathogen is seed borne fungus [20] [21], and can survive for at least two years on diseased stem tissues, plant debris, either on the soil surface or beneath [4].

The *C. destructivum* sporulates readily on infected cowpea at localized infection foci to produce anthracnose symptom within 96hrs of inoculation [22], in the form of lesions as small angular brown spots on the leaf petiole, the lower surface of leaves and leaf veins of cowpea grown under different cropping patterns [6]. These various spots created later coalesced to produce a brick red to brown discoloration of the entire leaf. Symptoms are usually delayed (delitescent infection) until production of flowering buds. This degree of virulence on cowpea often leads to product yield loss between 35% and 50% [8] [9].

Available management techniques for Anthracnose disease of cowpea include the use of Biocontrol systems (bioagents), pesticides (conventional/synthetic chemicals), cultural observations (clean seeds/hygienic fields and practices), HPR (host plant resistance) and botanicals (Biopesticides/no synthetic chemicals) [23]. Some, though seem effective, are enlaced with residual and often negative and indelible impressions [24].

The global quest for "back to nature" continues to augment the need and desirability to search for the alternative which employs natural agro-biological balance (Biopesticides) to address plant disease issues. This is remotely aimed at protecting the soil that supports the life of these crops among other horticultural plants [25], and in extension safeguards the environment for every other *vivo* organism.

The Environmental Protection Agency (EPA-USA) defines a Biopesticides as a pesticide derived from natural materials such as animals, plants, bacteria and certain minerals [26]. Biopesticides of plant origin are the botanicals (**Figure 1**). Biological control of plant disease through the use of antagonistic micro organism [4] [27]-[29] and botanical control of plant disease through the use of plant extracts [7]-[9] are two major ways in the control of plant disease in respect to natural agro biological balance.

In the evaluation of some botanicals against *C. destructivum*, Akinbode and Ikotun [4] inhibited the growth of the pathogen *in vitro* using *Nicotiana tabacum* plant extract. Crude botanical extracts from stem back and root bark of *Azadiractha indica*, *Vernonia amygdalina* and *Cochlospermum planchonii* exhibited strong fungi toxicity against *Colletotrichum capsici* as reported by Nduagu *et al*. [25].

Palhano *et al*. [30] inactivated spores of *Colletotrichum gloeosporiodes* using high hydrostatic pressure separate and combined with Citral or lemongrass (*Cymbopogon citratus*) essential oil. Their work reiterated the need for the use of plant essential oils as an alternative for crop health problems considering the safety and stability of the soil and its environment.

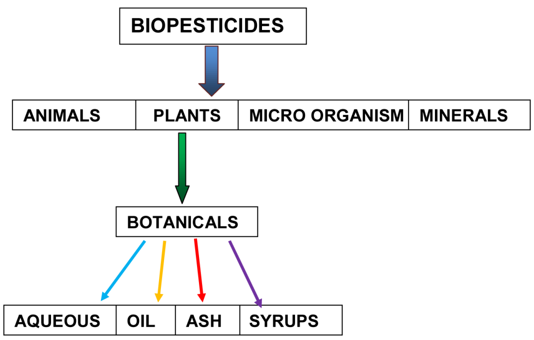

Figure 1. Botanicals in Biopesticides.

The search for bioactive substances from the plant world has led researches to distinctive regions of plants. Flowers, leaves, barks, seeds, fruits, roots and at times whole plant could be employed in the search for botanicals [31]. The seeds of neem, *Azadiractha indica* A. Juss and fruits of bush pepper, *Xylopia aethiopica* (Dunal) A. Rich were used in the work of Amadioha and Obi [8], while in another similar study the same authors employed the leaves of scent plant, *Ocimum gratissimum* (L.) and lemon grass, *Cymbopogon citratus* D. C. Stapf., for anthracnose disease of cowpea [32]. Amadioha [9] used the leaves of *Piper nigrum*, *Ocimum sanctum* and *Citrus limon* in his study against *C. lindemuthianum*.

Akinbode & Ikotun [4] and Colpas *et al*. [23] utilized the leaves of *Nicotiana tabacum* and *Ricinus communis*, and *Ocimum gratissimum* respectively in the scientific investigation for bioagents/botanicals in control of *C. destructivum*. Nduagu *et al*. [25] screened eleven plants on growth of *Colletotrichum capsici* (Synd) Butler & Bisby concentrating on leaves, stem bark and root barks of the concerned plants.

Different methods and techniques have been employed by scientists in the extraction and characterization of products from plants. A plant material could thus be harnessed in its fresh, or air/sun/oven-dried form, and with an adoption of extraction methods such as the use of hot or cold water for aqueous botanicals [4] [23] [25] [32] [33]; organic solvents for oil botanicals [8]; crude ashes for crude powder botanicals [34] [35]

There are weighty merits for the quest for wider exploration of Biopesticides in the field of agriculture. Biopesticides tend to pose fewer risks than conventional pesticides; Biopesticides are usually inherently less toxic than conventional pesticides; when used as a component of integrated pest management (IPM) programmes [25]. Biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high; Biopesticides require much less data and time frame to register than a conventional pesticide [36]. They are non residue producing control agent, making them eco-friendly and easy to use class of *Reduced-risk Fungicides*. It is in the support of all these that the European Community, like in other developed parts of the world, established a European Commission Working Document (SANCO/10472rev.5). This specifies data requirements for active substances of plants protection products made from plants or plant extracts.

2. Discussion

The global comparison of scientific research and publications, on protection of *Vigna unguiculata* (L.) (**Table 1**), projected the Asian region with the highest of 35.9%. This was followed by Africa with 24.80%. The least scientific research and publications on protection of cowpea was from Australia with 1.40%.Scientific documents on cowpea diseases according to pathogen groups, for five years range indicated the fungi to be on top with 35.6% (**Table 2**).This relatively high level percentage of scientific papers points at the major pathogenic constrains and its economic importance in the cultivation of cowpea crops. Regrettably the Biopesticides (Botanicals) which is a protective drive for a natural agro-biological balance in the fight against agricultural pests and disease was associated with about 7% of the total cowpea disease management options (**Figure 2**), a clear indication of under exploration of this area. This corroborates with the observation in the work of Emechebe & Lagoke [3].

It was observed that each cowpea pathogen has different regions of interest on a “whole cowpea plant”. This work, therefore, considered a cowpea crop from different botanical dimensions of six parts, and it was discovered that 30% of the fungal infections occur on the foliar part of the crop, 25% on the stems, 15% on the roots, 10% on the pods/fruits, 25% on the seeds/seedlings and 10% on the whole parts of the plant (**Figure 3**).

There was an indication that while the other nineteen pathogenic fungal species (**Table 3**) poses the ability each of attacking only about a meager 20% of a cowpea crop, *Colletotrichum* species especially the *C. destructivum* & *C. truncatum*, have in stock 100% virulence on a single crop each at a given pathogenic situation (**Table 3**). This corroborates with the findings of Latunde-Dada et *al.* [22], Latunde-Dada & Lucas [37], and Akinbode & Ikotun [4].

There are eighteen plant families presently under the anguish of the *Colletotrichum* Corda (**Table 4**). About 28% family interaction existed between plant families under the affliction of *Colletotrichum* and the plant families screened for antifungal properties (**Table 6**) as derived from **Table 4** & **Table 5**. These apparitions were observed within the five plant families of *Asteraceae*, *Caricaceae*, *Fabaceae*, *Lauraceae and Poaceae*. The rest of about 72% were unique in occurrence, hence no family interactions among them (**Table 6**.) The extrapolated values appear to affirm the indication on table 5 of this work that of all the entire plant families in existence only about eighteen (18) has been screened for their biofungicides characteristics between 1998 and 2011.

Table 1. Relative contributions, geographically, of scientific publication on cowpea diseases.

Geographical area.	Number of papers.	Percentage of papers contributed (%).
Africa	85	24.80
Asia	123	35.90
Australia	5	1.40
Europe	28	8.20
North America	75	21.80
S. America & the Caribbean	27	7.90

Source: [3].

Table 2. Publications, for five years, on cowpea diseases according to pathogen groups.

Group of pathogen	Number of papers	Percentage of papers (%)
Bacteria	21	6.10
Fungi	122	35.6
Nematodes	69	20.10
Parasitic plants	19	5.50
Viruses	112	32.7

Source: [3].

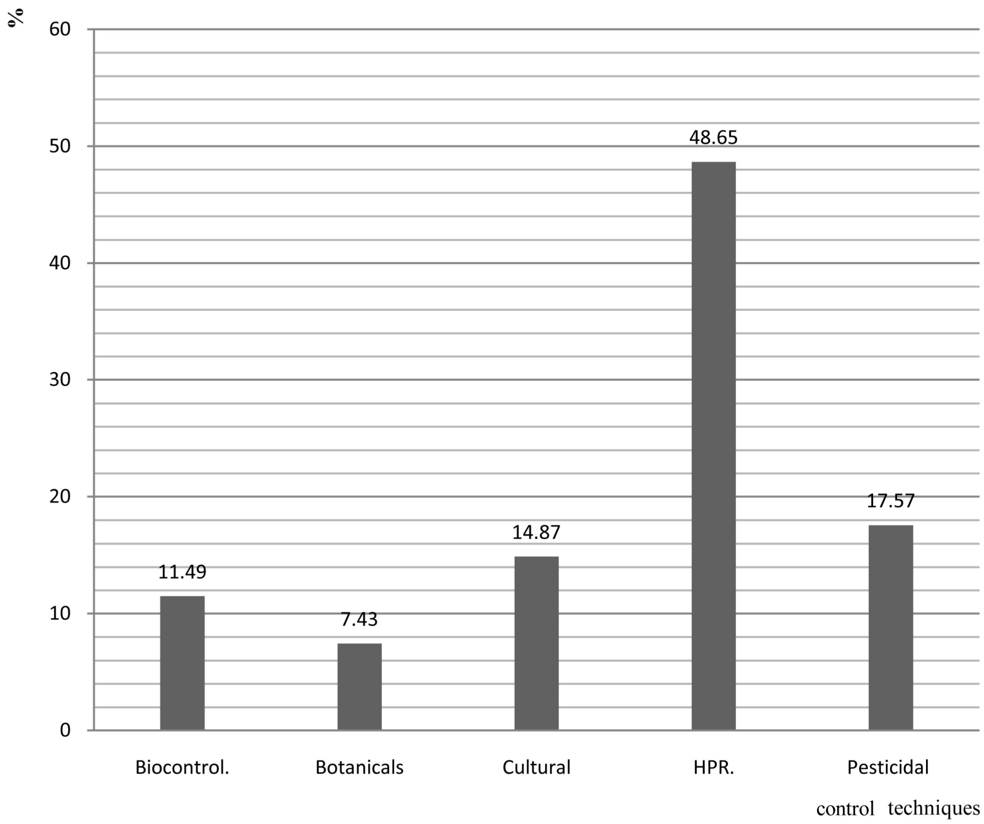

Figure 2. Percentage disease control techniques on *Vigna unguiculata* for a media decade.

The products screened during this thirteen years span were of various forms or state, such as (1) Aqueous: botanicals extracted using water as the solvent. The water also forms the extract solution [4] [25]; 2) Syrup: botanicals of a higher measure of viscosity having been extracted with a solvent other than water, and also containing some of the extracting liquid in its solution [23] [38]; 3) Oil: botanicals in the form of essential oil of the

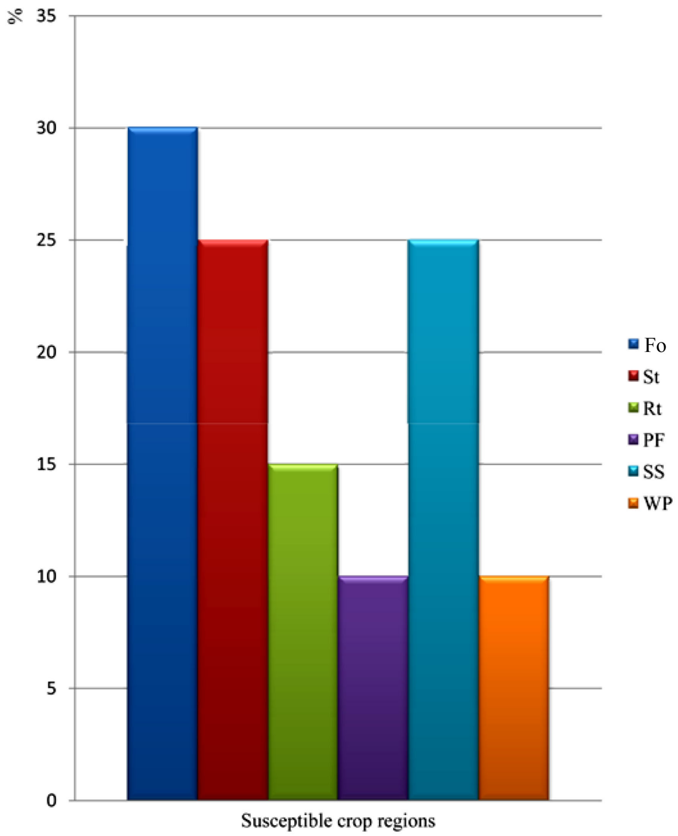

Figure 3. Percentage regional fungal attack on *Vigna unguiculata* crop. (Fo = foliar; St = stem; Rt = root; PF = pod/fruit; SS = seed/seedlings; WP = whole plant)..

test plants, usually extracted through a condensation system [8]; and 4) Ash: botanicals produced in the form of residues powder left after the combustion of a test plant material [34] [35].

Nevertheless, the botanical forms enumerated in this study could be extended to produce additional form by the application of further processing treatment on the original form. For example the Syrup produced in the work of Win *et al.* [38] was utilized in its dry crude botanical extract state after subjecting the initial extract syrup to an evapoconcentration system. This study, however, observed that most of the botanicals screened for this span of thirteen years (1998 to 2011) were produced and also utilized in their aqueous form. And considering the total 33 frequency occurrence of botanical forms (**Table 5**), the aqueous botanicals was 51.52%, followed by the syrup (15.15%), ash (18.18%) and the oil form of 15.15%. The relative easy and economy of production could be responsible for the high percentage value obtained with aqueous botanical evaluation.

3. Conclusions

Anthracnose disease remains a devastating health problem to cowpea crop and an equated hindrance to its economic cultivation. The major afflicting pathogen *Colletotrichum destructivum* O'Gara has the virulence of one hundred percent (100%) infection on a single crop stand (that is every part of the crop is subject to attack and infection by *C. destructivum* at a given pathogenic situation).

The use of botanicals remains suitable contest to adequate disease management options, at least for its characteristics ease of production, economy and ecological amiability.

This study has availed the fact that with the high global yearning for the urgent replacement of conventional (chemical) fungicides in disease management with the ecologically compatible bio-fungicides, the seemingly several works in this direction is merely about 7% of the different management systems as indicated on cowpea disease control options and therefore, advocates for more reconnaissance in this essential area of agro-biological

Table 3. *Colletotrichum* Corda and other fungal pathogens of *Vigna unguiculata*.

Pathogen	Disease afflicted	Region affected	Reference
Alteneria cassiae Juria & Khan	*Alternaria* leaf spot	Foliar	[39]
Cercospora canescens Ellis & Martin	*Cercospora* leaf spots	Foliar	[13]
Choanephora cucurbitarum (Berk & Rav.)	*Choanephora* pod rot	Pods/fruits	[15]
Colletotrichum dematium (Pers. ex Fr.)	*Colletotrichum* stem disease	Stem	[12]
Colletotrichum destructivum O'Gara	Anthracnose	Every part	[4]
Colletotrichum capsici (Syd.) Butl. & Bisb. (=*Colletotrichum truncatum* (Schw.)	Brown blotch	All parts	[22] [37]
Fusarium oxysporum f.sp *tracheiphilum*	*Fusarium* wilt	Seedlings	[17]
Fusarium oxysporum f. sp *vasinfectum* (E. F. Smith) Synd & Hans	*Fusarium* wilt	Seedlings	[40]
Macrophomina phaseolina	*Macrophomina* blight	Seedlings (severe mortality)	[41]
Mycospharella cruenta Latham. (Anormoph of *Pseudocercospora*)	*Pseudocercospra* leaf spots	foliar	[3]
Phomopsis longicola	*Phomopsis* pod spot	Pods/fruits	[16]
Protomycopsis phaseoli Ramak & Subram. (=*Entyloma vinae* Batista)	Leaf smut	Foliar	[14]
Pythium aphanidermatum (Edison) Fitz	*Pythium* soft rot	Stem	[11]
Pythium ultimum	Damping off (pre/post)	Seed/seedling	[18]
Phytophthora cactorum (Leb. & Chon.) Schroet.	Red stem canker	Stem/root	[10]
Phytophthora vignae Puress.	*Phytophtora* stem rot	Stem/root	[10]
Sphaceloma sp.(Anamorph of *Elsinoe phaseoli* Jenkin)	*Sphaceloma* scab.	Hypocotyls & epicotyls	[3]
Sclerotiumrolfsii Sacc. (Teliomorph: *Corticum rolfsii* Curzi).	Basal stem rot/wilt	Stem	[42]
Uromyces appendiculatus (pers.) Unger (=*U. vinae* Barclay).	Brown rust	Foliar	[43]
Thanatephorus cumeris (Frank) Donk (=*Rhizoctonia solani* Kuhn)	Web blight Rot (root rot /seedling disease complex)	Root/seedling	[3]

Table 4. Plant families under the affliction of Colletotrichum Corda (1995-2011).

Family	Reference.
Amaranthaceae Juss.	[44]
Anacardiaceae Lindl.	[45]
Asteracea Bercht. & J. Presl.	[27] [44]
Brassicaceae Juss.	[46]
Carcaceae Dumort.	[30]
Convovulaceae Juss.	[27]
Cucurbitaceae Juss.	[44]
Cuscutaceae Dum. (=*Convovulaceae*)	[27]
Fabaceae Lindl.	[3] [8] [22] [44] [47]
Lauraceae Juss	[45]
Leguminosae Juss., Non. Con (=*Fabaceae* Lindl.)	[1] [3] [6] [9]
Linaceae. L	[37]
Malvaceae Juss	[44]
Musaceae Juss	[44]
Oleaceae Hoffmgg.& Link	[48] [49]
Poaceae Barnhart (=*Gramineae*Juss, Non. Con.)	[27] [50]
Roasaceae Adans	[44] [48] [51]
Rubiaceae Linn	[52] [53]

Table 5. Plant families screened for biofungicidal properties (1998-2011).

Family	Samples	Bioassay	Botanical Form	Reference
Alliaceae	Garlic spp	*In vitro*	Syrup	[38]
Annonaceae	*Xylopia aethiopica*; *Annona reticulata*	*In vitro/In vivo*	Oil extract; Aqueous.	[8] [54]
Arecaceae	*Elaeis guineensis*; *Cocos nucifera*	*In vitro*; *In vitro*	Ashes; Ashes	[34] [35]
Asteraceas	*Chromoleana odorata*; *Vernonia amygdalina*	*In vitro*; *In vivo*	Aqueous; Aqueous.	[25] [33]
Caricaceae	*Carica papaya*	*In vivo*	Aqueous.	[33]
Cochlospermaceae	*Cochlospermum planchonii*	*In vitro*	Aqueous.	[25]
Euphorbiaceae	*Ricinus comunis*; *Hymenocardia acida*; *Euphorbia prostrata*	*In vitro*; *In vitro*; *In vitro*	Aqueous; Aqueous; Ashes	[4] [25] [35]
Fabaceae	*Tephrosia vogelii*; *Senna alata*	*In vitro*; *In vitro*	Aqueous; Ashes	[2] [35]
Lamiaceae	*Thymus vulgaris*; *Ocimum gratissimum*; *O. sanctum*	*In vivo*; *In vivo*; *In vitro*	Oil extract; Aqueous; Aqueous/Syrup	[23] [25] [32] [55]
Lauraceae	*Cinnamon zeylanicum*	*In vivo*; *In vitro*	Oil extract ;Syrup	[38] [55]
Meliciaceae	*Azadiractha indica*	*In vitro/In vivo.*	Oil extract; Aqueous	[8] [25]
Myrtacea	*Psidium guajava.*	*In vitro*	Aqueous.	[25]
Piperaceae	*Piperaceae*	*In vitro/In vivo*	Aqueous/syrup; Syrup	[9] [38]
Plumbaginaceae	*Plumbago zeylanica*	*In vitro*	Ashes	[35]
Poaceae	*Cymbopogon citratus*	*In vitro/In vivo*; *In vitro*	Aqueous; Oil extract	[30] [32]
Potederiaceae	*Eichhomia crassipes*	*In vitro*	Ashes.	[34]
Rutaceae	*Citrus limon*	*In vitro*	Aqueous/Syrup; Aqueous.	[9] [25]
Solanaceae	*Nicotiana tabacum*	*In vitro*	Aqueous.	[4]

Table 6. Spatial interaction between screened and pathogen afflicted plant families.

Plant family screened for biofungicidal properties(A)[1]	Plant family under the affliction of *Colletotrichum*(B)	Existence of spacial family interaction between A & B = C	Percentage family interaction [C/(AorB) x 100]
Alliaceae	*Amaranthaceae* Juss.		
Annonacea	*Anacardiaceae* Lindl.		
Arecaceae	*Asteracea* Bercht. & J. Presl.		
Asteracea Bercht. & J.Presl.	*Brassicaceae* Juss.	*Asteracea* Bercht. & J. Presl.	
Caricaceae Dumort.	*Caricaceae* Dumort.	*Caricaceae* Dumort	
Cochlospermaceae	*Convovulaceae* Juss.		
Euphorbiaceae	*Cucurbitaceae* Juss.		
Fabaceae Lindl.	*Cuscutaceae* Dum. (=*Convovulaceae*)	*Fabaceae* Lindl.	
Lamiaceae	*Fabaceae* Lindl.		
Lauraceae Juss	*Lauraceae* Juss	*Lauraceae* Juss.	**27.78%**
Meliciaceae	*Leguminosae* Juss., Non. Con (=*Fabaceae* Lindl.)		
Myrtaceae	*Linaceae* L.		
Piperaceae	*Malvaceae* Juss		
Plumbaginaceae	*Musaceae* Juss		
Poaceae Barnhart	*Oleaceae* Hoffmgg. & Link	*Poaceae* Barnhart	
Potederiaceae	*Poaceae* Barnhart (=*Gramineae* Juss, Non. Con.)		
Rutaceae	*Roasaceae* Adans		
Solanaceae	*Rubiaceae* Linn		

[1]Nominal value of A or B equals to 18; Nominal value of C is equals to 5.

system whose endpoint includes the provision of an eco-friendly global environment.

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