Potential of Botanicals to Control *Callosobruchus maculatus* (Col.: Chrysomelidae, Bruchinae), a Major Pest of Stored Cowpeas in Burkina Faso: A Review

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ABSTRACT: Cowpea is an essential food legume in the tropics and particularly for sub-Saharan African populations. Postharvest grain storage, however, is a major constraint for crop expansion and year-round availability due to the cowpea weevil, *Callosobruchus maculatus* F., the main storage pest of cowpeas in West Africa. The use of chemicals for cowpea storage is a common practice which represents, however, a risk for consumers, environment, and could also exacerbate pest control. In Burkina Faso, since the early 2000s, several scientific investigations have focused on the control of *C* maculatus using botanicals considered as promising and safe alternatives to chemicals. The aim of this review is to take stock of the research conducted and to identify the potential candidates on which future studies in this field will focus. The set of data analyzed show that several plants materials, including powders, crushed plants and essential oils (EO), were active against eggs, larvae, and adults of *C* maculatus, through dose-dependent mortality responses. However, EO extracted from native aromatic plants have yielded the most promising results, specifically EO from *Ocimum canum* appeared as the best candidate control agent. Other potentially interesting EO tested included *Hypitis suaveolens*, *Hypitis spicigera*, and *Lippia multiflora*. Based on these results, attempts to optimize the use of EO for cowpea storage were conducted in laboratory and field conditions. Side effects of botanicals toward the main biological control agent, the ectoparasitoid *Dinarmus basalis* have also been highlighted. The results are discussed in a view of practical use of botanicals and EO as safe alternatives for Integrated Pest Management in stored cowpeas in Africa and developing countries.

KEYWORDS: Botanicals, cowpea storage, essential oils, *Callosobruchus maculatus*, safe control methods, IPM

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Introduction

In West Africa, cowpea, *Vigna unguiculata* L. Walp., is a very difficult commodity to preserve, which compromises its optimal use as a nutritional supplement and as a considerable source of income for the most vulnerable populations.¹⁻⁴

Cowpea postharvest storage is constrained due to pod or seed infestation by bruchids among which *Callosobruchus maculatus* Fab. is the major pest.⁵⁻⁶ Bruchids’ attacks begin in the fields so that at harvest the seed infestation rate reaches 1% to 5%.⁶ However, this apparently low rate allows a residual larval ectoparasitoid *Dinarmus basalis* Rond. ²⁰,²¹ Research on hermetic storage has remained at the experimental stage with the identification of 2 potential natural enemies, the oophagous trichogram parasitoid *Usca naeophaga* Steph.,¹⁹ and the pteromalid larval ectoparasitoid *Dinarmus basalis* Rond.²⁰,²¹ Research on hermetic storage has led to the development of triple bagging technology with PICS bags.²² This technology is currently being widely used as an alternative to synthetic insecticides.¹²

Storage methods include the use of insecticides as common as they are considered by some to be cheaper and more effective than existing alternatives.¹²⁻¹⁴ Unfortunately, many side effects are associated with the use of chemicals which are hazardous to humans and environment,¹⁵ and which also exacerbate insect pest control due to resistance development.¹⁶ To address these chemical side effects, the search for alternatives has become a major challenge for scientific research and for consumer and environmental organizations.¹⁷,¹⁸ Several fields of research have been explored since the early 2000s in Burkina Faso, including mainly biological control, hermetic storage, and plant extracts. Although several natural enemies of *C maculatus* have been identified in both cowpea fields and stores,⁴ biological control has remained at the experimental stage with the identification of 2 potential natural enemies, the oophagous trichogram parasitoid *Usca naeophaga* Steph.,¹⁹ and the pteromalid larval ectoparasitoid *Dinarmus basalis* Rond.²⁰,²¹ Research on hermetic storage has led to the development of triple bagging technology with PICS bags.²² This technology is currently being widely used as an alternative to synthetic insecticides.¹²

Studying the effects of insecticidal or repellent plants for cowpea storage in Burkina Faso has received much attention.

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The numerous results obtained, although variable, indicate that there is a potential to be valued after several years of research mainly prospective. To achieve this, an analytical synthesis of the results and a reframing of the research are needed to optimize the practical use of plants that have already proved their effectiveness. The present review of the potential of the plants to control *C. maculatus* is in this context. It summarizes the research undertaken since the 2000s, focusing on the best plant candidates on which research perspectives should now focus.

**Economic Importance of *Callosobruchus maculatus* Fab**

*Callosobruchus maculatus* Fab. is a beetle that belongs to the family Chrysomelidae and to the subfamily Bruchinae. This insect is commonly known as cowpea weevil or bean beetle because several stored legumes are attacked.24 Females lay eggs on seed coat and larvae develop exclusively inside the seeds, at the expense of grain endosperm and embryo, and are responsible for cowpea damage.8 Several generations of flightless form *C. maculatus* can therefore overlap in stocks during cowpea postharvest storage in West Africa.8 The infested seeds become increasingly hollow resulting in weight loss and perforation, adult insect emergence holes at the end of larval growth. Therefore, *C. maculatus* is considered as the most important storage pest of cowpea throughout the tropics.25

The quantities of cowpeas lost annually are high despite the fact that precise data are not available, mostly expressed as percentages.26 These losses were estimated annually at 2.4% per ton of cowpea pods in storage in Niger.27 In Nigeria, losses ranging from 10% to 50% during storage were also reported.28 Moreover, farm storage for 6 months was accompanied by 70% seed infestation and about 30% weight loss and virtually unfit for consumption.29 These losses are correlated with economic losses as seed quality is an important determinant of market prices.10 For a good assessment of economic losses, a model based on cowpea weight loss caused by individual *C. maculatus* in the establishment of an economic injury level of *C. maculatus* on cowpea has been developed.20 However, reliable data on economic losses on cowpea due to *C. maculatus* are rare and deserve further investigation.

**Side Effects of Synthetic Insecticides Used for Grain Preservation**

Synthetic insecticides have played a historical role in agriculture development in general and particularly in the postharvest grain storage.17,30,31 However, their use by farmers is criticized worldwide.12,32,33 Although the importance of insecticides in storage pest control has been clearly established,33 it remains that in the African context, the misuse of chemicals exposes human populations to health hazards12,34,35 and negatively affects the environment.36,37

Health risks are mostly correlated with lack of training and information for insecticide users, poor selection of insecticides, overdose when applying, and inadequate use of protective equipment when handling pesticides.12,38–40 To illustrate this, a survey of cowpea traders in Burkina Faso found that 77% of the insecticides used were neither registered nor intended for food preservation.12 Similarly results from another study showed that traders consider the insecticide-treated grains to be fit for resale and therefore safe for consumption if they no longer spot traces of applied chemicals on the treated grains and to a lesser degree if they do not perceive the odor of the pesticidal materials on it.11 Thus, there is no due regard for waiting period vis-à-vis the dosages applied with no reasonable allowance, made for effective biodegradability of these chemicals to less harmful constituents. The effects of exposure to pesticides include development of many cancers as well as the risk of genotoxic, immunotoxic, and neurotoxic and adverse reproductive effects and an increased incidence of psychiatric and dermatologic conditions.41,42

Environmental risks are mainly related to the storage and application of insecticides in inappropriate locations and also to empty packaging disposed of in environment after use.12,43 One of the major problems caused by the excessive use and/or misuse of synthetic insecticides is the advent of resistant strains within the treated pest populations, which results in the difficulty of controlling such insects. Resistance to various insecticides has been reported in several stored-product insects in the world.44–46 The most frequently cited insect species include *Sitophilus zonais*, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, and *Oryzaephilus surinamensis*. All of these pests are known to be resistant to phosphate used in the fumigation of grain stocks. Previous studies reported on a population of *T. castaneum* that was 119 times more resistant to phosphate and 3 populations of *R. dominica* that were 254, 910, and 1519 times more resistant than the susceptible population found in insects collected from commercial grain storage structures in Oklahoma.46 Studies of *C. maculatus* resistance to chemicals are rare, but the frequency and overdose of the use of fumigants in cowpea storage are factors favoring resistance to this type of insecticide.12 Authors studying resistance of *C. maculatus* to pirimiphos-methyl in 3 zones in Nigeria concluded that insect age and origin influence their susceptibility to that insecticide.45 Diverse levels of resistance to dichlorvos were also observed in Nigerian populations of *C. maculatus* depending on their origin.47 All these potential side effects make it imperative to search for alternatives to chemicals.

**Plant Species With Insecticidal Potential to Control *C. maculatus* in Burkina Faso**

The use of plant material for grain preservation and particularly cowpea is considered as a promising alternative to synthetic insecticides for several decades.48 Plant material may produce volatile chemicals that repel or confuse the adult beetles, eventually preventing invasion or causing emigration from treated stocks.48 Some other plants produce secondary
metabolites that directly affect development and reproduction of storage pests. In this respect, several studies have been conducted on the effects of plants with insecticidal potential to control the cowpea beetle. In Burkina Faso, several plant species have been involved in these studies since the early 2000s (Table 1 and related references). Extensive studies were conducted on 6 plant species from 3 families including Capparaceae, Lamiaceae, and Verbenaceae, through bioassays on C maculatus, cowpea storage trials and side effects of botanicals on biological control agents (Table 1).

Potential of Botanicals to Control C maculatus
The results achieved from the evaluation of the insecticidal and/or repellent potential of the plants tested against C. maculatus can be grouped into 2 parts: bioassays and cowpea storage trials. Three major types of plant materials including powders or crushed plant material and essential oils (EO) have also been tested.

Results from bioassays
The overall results on the effects of powders or crushed plants and of the EO tested are summarized in Table 2.

The powders and crushed plants generally have little effect on mortality of C maculatus adults, with some exceptions. Fresh crushed leaves of Boscia senegalensis and Cleome viscosa resulted in dose-dependent mortality, with 100% mortality reached in 24 hours of exposure to 4 and 76.9 g/L for B senegalensis and C viscosa, respectively. Interestingly, both plant species belong to the same family of Capparaceae, which may explain their particular efficacy in the raw state. It has been shown that the active compound of the leaves of B senegalensis is methyl isothiocyanate (MITC), a compound very toxic to insects. Ocimum canum dry powder causes more than 90% mortality of C maculatus adults at a dose of 3 g/L, with total mortality at 10 g/L after 24-hour exposure. This result is all the more surprising given that, under the same experimental conditions, powders from other Lamiaceae (Hyptis spicigera, Hyptis suaveolens) and Verbenaceae (Lippia multiflora) have been reported having no effect on adults even at the highest doses tested.

However, the powders and crushed leaves of all the plants tested exhibited ovicidal and inhibitory effects on the egg-laying of C maculatus females. The ovicidal effect, as well as the inhibition of egg-laying, is dose-dependent and appears to be more important with fresh crushed leaves of B senegalensis and C viscosa. A total mortality of the eggs exposed to 24 g/L of crushed B senegalensis leaves was observed, whereas the same result was obtained only at a dose of 76.9 g/L after 48-hour exposure to crushed C viscosa.

The dry powders of O canum, H spicigera, H suaveolens, and L multiflora have >50% ovicidal effects at 48 hours of exposure without ever reaching 100%.
It has also been shown that plant powder can exert a repellent effect on *C. maculatus* adults. Studies conducted in a Y-olfactometer with very low doses of powder of *H. spicigera* and *H. suaveolens* made it possible to obtain respective repulsion indexes of 0.63 and 0.56 *P > .05*. Finally, there were no significant effects on the larvae and pupae of *C. maculatus* exposed to doses ranging from 1 to 10 g/L of powders, except an unusual increase in the duration of development time. Under the experimental conditions (temperature varied 26°C-28°C and relative humidity was in average 30%), development time increased from 34.5 ± 0.3 days in the absence of plants to, respectively, 48.8 ± 6.5 days in the presence of 20 g/L powder of *C. viscosa* from 34.5 ± 0.3 days in the absence of plants to, respectively, 54.7 ± 6.6 and 63.9 ± 2.8 days in the presence of 20 g/L powder of *B. senegalensis* from 34.5 ± 0.3 days in the absence of plants to, respectively, 63.9 ± 2.8 days in the presence of 20 g/L powder of *C. viscosa* and 6.9 ± 2.5 days in the presence of 20 g/L powder of *B. senegalensis*.

Authors studying the persistence of EO and the effect of temperature on the maintenance of their biological activity showed a difference in the persistence of EO in hermetic natural conditions, *O. canum* being the most persistent and remaining 100% active for at least 14 days. However, a larvicidal effect was demonstrated using 4 g/L of crushed *B. senegalensis* leaves on both young (L2) and elder (L4) larvae.

More significant effects were obtained from testing the EO of several aromatic plants on adult survival, egg-laying inhibition, survival of eggs, larvae and nymphs, and finally repulsion of adult *C. maculatus*. Thus, all the EO tested were found to be toxic to adults with LC50 values of 0.23, 1.30, 5.53, and 6.44 μg/L, respectively, for *O. canum*, *H. suaveolens*, *H. spicigera*, and *L. multiflora*. Under the same conditions, the LC50 values were higher on the eggs of *C. maculatus* (LC50 ranged from 14.27 to 31.69 g/L), which reflects a greater tolerance of eggs compared to adults. It is surprising that the toxicity of the same EO varies completely depending on the developmental stage of the exposed insect. It has been shown, for example, that the most effective EO on eggs is *L. multiflora* (LC50 = 14.27 μg/L), whereas it is the least toxic for adults.

In order to determine the potential persistence of EO in stored cowpeas, laboratory trials have been conducted. These trials have shown that the EO of the above-mentioned plants can influence the degradation of EO. The repellency indexes calculated for *H. spicigera* and *H. suaveolens*, respectively, of 0.78 and 0.92 are significantly higher than those obtained with the powder of the same plants (*P < .05*). The biological activity of EO is generally due to a set of volatile compounds mainly comprising mono- and sesquiterpenes (Table 3).

Table 3. Major chemical compounds found in different plant materials tested in Burkina Faso.

| PLANT SPECIES         | PLANT MATERIAL TESTED | MAJOR COMPONENTS                        | REFERENCES |
|-----------------------|-----------------------|------------------------------------------|------------|
| *Boscia senegalensis* | Crushed leaves        | Sulfur containing compounds (methyl isothiocyanate) | 58         |
| *Cleome viscosa*      | Crushed leaves        | Flavonoid; phenols                       | 65         |
| *Ocimum canum*        | Powder                | nd                                       | 66         |
| *Hyptis suaveolens*   | Powder                | nd                                       | 67         |
| *Hyptis spicigera*    | Powder                | nd                                       | 68         |
| *Lippia multiflora*   | Powder                | nd                                       | 69         |
|                       | Essential oils        | 1-8 cineole; camphor; cis-, trans-piperitol |            |
|                       | Essential oils        | β-caryophyllene; sabinene; 1,8-cineole   |            |
|                       | Essential oils        | α-pinene; β-caryophyllene                |            |
|                       | Essential oils        | Thymol; p-cymene; thymol acetate         |            |

Cowpea storage trials

Laboratory bioassays do not take into account the actual conditions of cowpea storage. To mitigate this limit some studies have also addressed long-term storage issues in conditions as close as possible to on-farm storage, either in a controlled laboratory environment or on-farm. Studies have yielded variable results which tend to confirm some potential of protecting stored cowpeas using crushed *C. viscosa* and *B. senegalensis*. The introduction of 5
to 25 g/kg of crushed C viscosa into batches of 20 kg cowpea stocks at the beginning of storage has been shown to reduce the impact of C maculatus by 36% to 87% after 4 months of storage.52 Similarly, the introduction of 500 g of B senegalensis crushed leaves in granaries at the beginning of storage has made it possible to protect batches of 3 kg of cowpeas for at least 3 months. However, in the latter case, if storage lasted for 6 months, this effect no longer appeared suggesting a loss of activity after the first 3 months of storage.59 These results should serve as the basis for optimizing the use of C viscosa and B senegalensis to protect stored cowpeas. The interest of such results based on the use of raw plant material is that this practice could be easier to adopt by farmers and end users.

Storage trials were also conducted using EO.53,57 These studies have shown that several factors can affect the effectiveness of EO in stored cowpeas. Thus, the efficacy of H spicigera and L multiflora EO is dependent on the amount of cowpea seed treated, whereas this factor does not influence the activity of O canum and H suaveolens EOs.57 Using up to 7 applications of EO over a 4-month period of cowpea storage, these authors also showed that the efficiency of EOs does not depend on the number of applications but on its specificity on their chemical composition. It was also shown that not all cowpea storage containers potentiate the activity of EOs as plastic containers are more suitable than aluminum ones. Finally, the aromatization of powders of kaolin, starch, and diatomaceous earth with EO made it possible to obtain insecticidal powders just as effective as pure EO.57

### Side Effects on Biological Control Agents

Plants with insecticidal activities are often wrongly considered to have no adverse effects on humans and nontarget animals without this being verified. Because their use is considered as an alternative to hazardous synthetic insecticides, it should be verified that these plants have no side effects or that, where they exist, they can be mitigated. In addition, any approach to controlling insect pests should also be part of an integrated management strategy.59 It is the reason why most of the plants tested in Burkina Faso against C maculatus have also been evaluated on D basalis, an ectoparasitoid of bruchid larvae known as an excellent biological control agent.20,21

In general, the results demonstrate important unexpected effects of powders and EO of plants tested on D basalis populations53,58 (Table 4). Specifically, it has been shown that the EO of O canum, H spicigera, H suaveolens, and L multiflora have a significant acute toxicity to adults of D basalis and this effect was greater than that obtained on the host, C maculatus.53 Similarly, it was demonstrated that D basalis was more affected by treatment using MITC, a sulfur-containing compound released by B senegalensis leaves than its host C maculatus.58 From this latter result, it can be assumed that the introduction of B senegalensis leaves releasing MITC in the storage systems will reduce the density of the parasitoid population and so increase the seed losses by permitting the development of the bruchid population.

Investigating the role of plant material–based treatments in the behavior of parasitoids, studies have been conducted to determine the influence of sublethal doses of powders and EO of H suaveolens on host location behavior by D basalis females.62 Olfactometer studies showed that sublethal doses of volatiles emitted by the powders and EO were repellent for naïve females D basalis, ie, females which had previously developed in the absence of H suaveolens volatiles. Their reproductive activity was consequently reduced. However, females, which had been exposed to sublethal doses of H suaveolens volatiles during their postembryonic development, were no longer repelled or only partially repelled by the plant volatiles.62 A habituation process may be involved in the behavior of these D basalis females. The role of such a habituation process on the survival of parasitoids and the integrated management of treated stocks remains to be more precisely determined.

Previous studies also investigated whether grain protectants from H spicigera and H suaveolens (Lamiaceae) disturb parasitism and postembryonic growth of the parasitoid. They concluded that both plant species exert acute toxicity on D basalis larvae and also act as growth inhibitors.63 The same authors also showed that when cowpeas containing bruchid larvae were treated before being placed in the presence of D basalis females,

### Table 4. Diversity of side effects of different plant materials on Dinarmus basalis, an ectoparasitoid or bruchid larvae/pupae53,58,62,63

| TYPE OF EXPERIMENTS | PLANT MATERIALS | SIDE EFFECTS |
|---------------------|-----------------|--------------|
|                     |                 | TOXICITY     | PARASITISM ABILITY | POSTEMBRYONIC DEVELOPMENT | BEHAVIOR   |
| LC50 determination  | Methyl isothiocyanate (Boscia senegalensis) | Adults and larvae |              |                  |            |
|                     | Essential oils of Ocimum canum, Hyptis spicigera, Hyptis suaveolens, and Lippia multiflora | Adults and larvae |              |                  |            |
| Sublethal doses applied | Powders and essential oils of H spicigera and H suaveolens | Reduction Inhibition Repellence and habituation process |

Gray boxes = no data available.
the rate of parasitism decreased on average up to 24% and 47% in the presence of leaf dry powder and EO from *H spicigera* and *H suaveolens*, respectively.

Finally, the few cowpea storage trials conducted under natural conditions of grain infestation showed some incompatibility of the use of *B senegalensis* crushed leaves with that of parasitoids *D basalis* as biological control agent.\(^59\) Indeed, in situations of simultaneous combination of both control components, one did not obtain a summation of the impact of each of them considered separately.\(^59\) The results obtained in this section show globally that the parasitoid *D basalis* is more sensitive than its host *C maculatus* to the plant materials used to protect cowpeas. However, it is interesting to note that because storage devices are generally confined environments, the survival and behavior of *D basalis* will depend on its ability to adapt and exploit its hosts. The habituation process demonstrated that *D basalis* is capable of adaptation in an environment treated with plant material, suggesting possibilities for integrated pest management combining plant-based treatment with releases of parasitoids *D basalis*.\(^62\) However, the conditions for such a harmonious combination are not yet known and therefore deserve to be determined. This is all the more important because in natural conditions, cowpea seeds harvested and stored often contain both bruchids and parasitoids, which makes possible a direct effect of the insecticidal plants introduced into the granaries on the parasitoid population.

**Perspectives for Optimizing Botanicals for Cowpea Storage**

The results presented in this review show that there is an interesting potential to be exploited from the tested plant materials. The use of plant powders as raw material seems uninteresting, except for those of 2 plant species: *C viscosa* and *B senegalensis*. However, their optimal use rely on a more accurate determination of effective doses for large-scale storage. The advantage of such a practice is that it is already known to cowpea producers and that an optimal use strategy, if available, could easily be adopted. However, if the efficacy doses are very high, as shown by some laboratory results, this will have a damaging effect on the management of natural resources, i.e., availability of plants in nature, unless a plant production strategy is developed and extended.

In keeping with the numerous previous studies,\(^50,72,73\) EO have a double advantage in that they are not only more effective than raw plant materials but also are in low doses. The biological activity of these oils is mainly based on their chemical composition and probably on a synergy of action between the numerous compounds contained in each of them. One of the most effective EO is that of *O canum* which has been remarkable in all the studies conducted. It is mostly composed of 1,8-cineole,\(^65\) a compound with previously known biological activity against many storage insect pests.\(^74\) Further investigations should be conducted with this EO to optimize its potential. The option of aromatizing powder media does not seem to affect the biological activity of the EO. This procedure makes it possible to obtain insecticidal powders which can be used as synthetic insecticides. Such formulations reduce the volatility of the EO and increase their persistence. Further studies in this area should make it possible to identify the best candidate powders for aromatization. It is also important to continue research on the chemical composition of the EO tested to identify the active compounds as well as to assess their safety for humans and animals. Thus, depending on the chemical composition of each EO, it could be easy to anticipate whether it is appropriate for the preservation of foodstuffs. The chemical composition of an EO is very complex and subject to many variables related to plant organs used, harvest seasons, climatic and edaphic conditions, chemotypes.\(^75\) A review referring to 230 aromatic plants from a wide geographic distribution shows that the main compounds of EO are terpenoids (mono- and sesquiterpenes) to which are added some aromatic compounds.\(^76\) However, the biological activity of an EO is usually attributed to one or more dominant compounds.\(^76\) Studies on the long-term effects of EO on treated insects will better help to manage the development of possible resistance in pests treated with EOs. Finally, the socio-economics of botanicals and EOs are of great interest and deserve to be clarified as this will determine their availability and accessibility to users.

**Conclusions**

Studies conducted in Burkina Faso since 2000s on the insecticidal and/or insect-repellent potential of 6 plant species belonging to 3 different plant families have produced numerous results. The issues covered by these studies included the evaluation of the biological activity of powders, crushed leaves and EO on *C maculatus*, major pest of stored cowpeas and its parasitoid *D basalis*, induced repellant effects, and treatment of cowpea seeds in laboratory experimental storage situations and on a large scale. Plant material proved to be insecticides, active on *C maculatus* adults and eggs, with effects depending on plant species, material, and doses used. They were also repellent and inhibited egg-laying. The best candidates for use as raw plant material (powders or crushed plants) are *Cleome viscosa* and *Boscia senegalensis*, both plant species belonging to the Capparaceae family. However, the conditions for their optimal use remain to be determined. The best *C maculatus* control potentialities are offered by EO extracted from the 4 aromatic plants species tested. These highly volatile compounds could be formulated as insecticidal powders obtained by aromatizing starch, kaolin, or diatomaceous earth. Studies are still needed to optimize the use of EO, specifically addressing the persistence of aromatized powders, their safety to humans and nontarget organisms, their potential for inducing resistance in the pest, and their contribution to implementation of a global integrated management strategy to preserve stored cowpeas.
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Author Contributions

The idea for this review and the drafting plan were proposed by AS who presented a first version of this paper to the second conference on pesticidal plants held on 6–9 February in Victoria Falls, Zimbabwe. All co-authors participated in the compilation of the scientific information and the finalization of the manuscript.

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