Ageratum conyzoides L. for the management of pests and diseases by small holder farmers.

Naomi B. Rioba[1], Philip C. Stevenson[2,3*]

[1]School of Agriculture and Biotechnology, University of Kabianga, P.O. Box 2030-20200, Kericho, Kenya

[2]Natural Resources Institute, University of Greenwich, Chatham Maritime, Kent ME4 4TB, United Kingdom

[3]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, United Kingdom

*Corresponding Author: p.c.stevenson@greenwich.ac.uk.

ABSTRACT

Ageratum conyzoides L. (Asteraceae) is an aromatic, annual herb and cosmopolitan weed commonly known as billygoat weed or goat weed and is typically found in cultivated fields and other disturbed ecosystems. The species has been studied widely owing to its biological properties and its potential application in medicine and agriculture. Due to its importance and use in the treatment of burns and wounds, arthrosis, malaria, asthma, leprosy and dermatitis, its medicinal properties have been reviewed. *A. conyzoides,* however, also has insecticidal activity against a range of major pests of field crops and stored products including *Callosobruchus chinensis* L. (Coleoptera: Chrysomelidae), *Chilo partellus* Swinhoe (Lepidoptera: Crambidae), *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Panonychus citri* McGregor (Tetranychidae, Panonychus), *Sitophilus zeamais* Motchulsky (Coleoptera: Curculionidae), *Plutella xylostella* L. (Lepidoptera: Plutellidae) and *Brevicoryne brassicae* L. (Hemiptera: Aphididae). Evidence suggests that its efficacy is comparable to synthetic pesticides and that it is economically viable too while its impact on beneficial insects including ladybirds, hoverflies and spiders is much reduced compared to synthetics. Anti-microbial activity against important agricultural disease agents is also reviewed here including against *Fusarium oxysporum* Schltdl., (Hypochreales: Nectriaceae), *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) Leonian, (Phythiales: Phythiaceae), *Pythium aphanidermatum* (Edson) Fitzp., (Peronosporales: Pythium), *Fusarium solani* Mart (Sacc.) (Hypochreales: Nectriaceae) and *Gibberella zeae* (Schwein.) Petch (*Fusarium graminearum* (Schwabe) (Hypochreales: Nectriaceae). These activities suggest a compelling multipurpose plant that has merit as a potential commercial species. Since earlier reviews have focused on medicinal properties of *A. conyzoides* and less on its potential value in agriculture, this review seeks to bridge this gaps by reviewing research on the various properties of this species that are relevant to food production. The review presents updated information on the use of this species as an agricultural resource and emphasizes its potential as an industrial crop.

Keywords: *Ageratum conyzoides*, insecticidal, antifungal, nematicidal, herbicidal, ecosystems

1.0 Introduction

Small holder agriculture is defined as that which involves cultivation on less than 2 hectares of land and ownership of only a few heads of livestock (Salami et al., 2010). It employs 500 million people in the developing world and provides 80% of the food production to these countries (UNEP, 2013, FAO, 2010). Most African agriculture takes place on small-scale with median size of crop farm being between one and two hectares in most of the countries (Eastwood et al., 2010). The smallholders engage in very intensive farming than large farms (Lipton, 2005) resulting in high levels of productivity per unit of land (Barrett et al., 2010; FAO, 2014b; Larson et al., 2014; van Zyl et al., 1995; Binswanger-Mkhize and Mc Calla, 2010). However, small holders are typically resource poor and access to technologies to control the pests and diseases that limit production and successful storage is often lacking (Stevenson and Belmain, 2016). Synthetic pesticides still offer the primary tool for controlling biological constraints but are not always available particularly in remote areas. They may also be adulterated by unscrupulous traders, have serious adverse effects on non-target species (Isman, 2006; Ntow et al., 2008), pose significant poisoning risks to farmers and consumers, are expensive and their incorrect use (Ntow et al., 2008; Coulibaly et al., 2007) by illiterate farmers increases the chances of resistance building up in pest populations (Rathi and Gopalakrishman, 2006; Obeng-Ofori and Ankrah, 2002; Sharma and Meshram, 2006; Huang et al., 1999; Okonkwo and Okeye, 1996; Wei et al., 2013; Ogendo et al., 2003; Brent and Hollomon, 1998; Dubey et al., 2007; Kumar et al., 2007). Botanical insecticides and 'basic substances' (as defined under Regulation (EC) Nº 1107/2009) from plants have fallen short of the commercial potential that was predicted in the 1980s and 1990s (Isman, 2006). However, their use in small holder farming is likely to be the most significant route for their large-scale use and uptake (Isman, 2008). Plant species offer potential as materials that could be used in crudely prepared forms or as basic substances but very few e.g., Neem [*Azadirachta indica* (A. Juss.) (Sapindales: Meliaceae)] and Pyrethrum [(*Tanacetum cinerariifolium* (Treviranus) (Sch. Bip.) (Asterales:Asteraceae)] have any commercial track record (Isman, 2006). However, plants species continue to be the focus of much study for their pesticidal properties (Isman and Grieneisen, 2014) with increasing interest in Africa. One species that is of interest and has received much attention from researchers is *Ageratum conyzoides* L. (Asteraceae). *A. conyzoides* is an aromatic, annual herb which grows to a height of 1 M. It is native to Central America but is invasive globally and particularly in Southeast Asia, South China, India and West Africa (Iwu, 2000; Prince and Prabakaram, 2011; Amadi et al., 2012) and is especially successful in disturbed areas including agricultural land, roadsides, natural and planted forests, range/grasslands, riparian zones, water courses, wetlands and wastelands (Nasrin, 2013; Osho and Adetunji, 2011; Smith, 1991; Swarbrick, 1997).

Plant secondary metabolites provide plants with benefits through their accumulation in plant tissues for defence against herbivores, fungi and bacteria and as plant signals (Schoonhoven et al., 2005). These biological activities can be exploited for various human uses and in this respect *A. conyzoides* has considerable potential. The present review provides an update on *A. conyzoides* chemistry and human use with a strong emphasis on its applications in food production. We argue that this species has potential as a non-food crop and we predict considerable scope for its use in pest control. In addition, we propose that the species could support crucial ecosystem services of both pollinators and natural enemies of pests and so have multiple benefits, particularly in small holder agriculture.

2. *Ageratum conyzoides* chemistry, phytotoxicity and product formulation.

Numerous studies report on the chemistry of *A. conyzoides* which is essential to inform the development and optimization of new uses for this plant. The species produces a diversity of compound classes with examples of those most reported presented in Figure 1. Conyzorigum, a cromene was identified by Vyas and Mulchandani (1984) while Borthakur and Buruah (1987) identified Precocene I and II. In addition to Precocene I and II, Ekundayo et al. (1988) identified 51 terpenoid compounds in the whole plants. Gonzales et al. (1991) reported 11 chromenes in essential oils of the plant with 6-angeloyloxy-7-methoxy-2, 2-dimethylcromene being published for the first time, while two more chromenes, ageratocromene and β-caryophylene have also been reported (Vera, 1993). Other compounds reported were flavones including ageconyflavones A, B, and C (Vyas and Mulchandani, 1986) and hexametoxyflavones (Horie et al., 1993) along with 2H-chromen-2-one (Ladeira et al., 1987). The species also contains alkaloids, mainly pyrrolizidine group for example 1, 2-desifropirrolizidinic and licopsamine (Trigo et al., 1988; Weindenfeld and Roder, 1991).

However, this review will focus primarily on the significant potential use of this species in agriculture particularly through the insecticidal activity but also herbicidal, anti-microbial and nematicidal activity all of which may play a valuable role in the agricultural economy. Issues that necessitate the search for cheaper pesticides have been presented above but one important additional consideration is that synthetic insecticides are broad spectrum and are more harmful to beneficial insects than plant based pesticides including *A. conyzoides* (Amoabeng et al., 2013). Similarly, most fungicidal agents in the market are toxic and have undesirable effects on other beneficial organisms present in the environment such as natural enemies of pests and pollinators (Choi et al., 2004). The level of toxicity of botanical pesticides has also been questioned because some of the chemical compounds in such products may result in toxicity to humans or to the crops. There is conflicting information regarding the toxicity of *A. conyzoides*. Some researchers (e.g., Trigo et al., 1988) reported several alkaloids including 1,2-desifropirrolizidinic and licopsamine which can have hepatotoxic activity. Abbiw (1990) reported that the plant was poisonous to rabbits due to the presence of coumarin and hydrogen cyanide. It was further reported by Bosi et al. (2013) that pyrrolizidine alkaloids were hepatotoxic and can cause acute poisoning. Contrarily, however, several studies have found *A. conyzoides* to be non–toxic and thus safe (Moura et al., 2005; Arya et al., 2011; Diallo et al., 2010). Antai et al. (2009) showed that ingestion of the extract at 300, 400 and 600 mg/kg body weight may not be toxic to humans. Similarly, Igboasoiyi et al. (2007) indicated that ethanol extract of *A. conyzoides* at a dose of 500 to 1000 mg/kg body weight, orally administered daily for 28 days did not have any detrimental effects on the liver, kidney, bone and pancreas of rats, concluding that the extract was safe for use in ethno-medicine. Furthermore, Ita et al. (2009), Atoui et al. (2005) and Hatem et al. (2010) showed that the plant even has hepatoprotective effects. While conducting clinical trials with patients with arthrosis, Marques-Neto et al., (1988) administered an aqueous extract of the whole plant and they reported analgesic effect in 66% of patients and improvement in articulation mobility in 24 % without side effects. Thus, while there is some conflicting opinion in the literature the greatest weight of evidence suggests *A. conyzoides* is low risk.

More evidence for its safety is given by the fact that in Brazil, its aerial parts are used widely (both internally and externally, fresh or dried, in tinctures or infusions) for their reported analgesic and anti-inflammatory properties, and are also commonly used to treat menstrual cramps, arthritis, rheumatism, and diarrhea (Okunade, 2002; Lorenzi and Matos, 2008). Such well-established popular use of this plant in Brazil, led to its inclusion in the list of notified herbal drugs, a category of medicinal crude drugs created by the Brazilian Health Surveillance Agency, Anvisa (RDC No. 10, March 9th, 2010). This means the crude drug (aerial parts, crushed or powdered) is now authorized for marketing without medical prescription, for use in the preparation of infusions. In fact this led to increased demand for this plant triggering research on cultural practices (Magalhaes et al 1989; Correa et al., 1991; Ming, 1998) in the effort to commercialize its production.

Secondary metabolites play an important role in the protection of plants as antibacterial, antiviral, antifungal and insecticidal agents (Hajlaoui et al., 2009) and so their use as an alternative solution to the environmental problems caused by synthetic pesticides is valid and many researchers are trying to identify effective natural products to replace synthetic chemistries (Kim et al., 2005; Isman, 2006; Isman and Grieneisen, 2014). Plant compounds are typically degraded rapidly in sunlight and in soil limiting their impact on the environment so they can have an effective role in sustainable agriculture (Cho et al., 2006).

Because plant compounds are quickly degraded, for them to be effective in plant pest and disease control, there is need to develop appropriate formulation. However, the literature provides very limited information on progress made in the formulation identifying a research gap. That said, Prajapati et al. (2004) reported an emulsifiable concentrate formulation using methyl oleate (biodegradable solvent) to avoid toxicity of the formulation. They further noted that extraction from the plant using methanol as a solvent gave good yields and that the emulsifiable concentrate of these extracts was optimized with the help of methyl oleate as a solvent and mixture of non-ionic emulsifiers. They indicated that these extracts as well as their formulation possess antimicrobial activity that can be used for agricultural purposes. However, this kind of formulation may not be suited to a small-scale farmer hence the need for further research to come up with the best formulation for adoption by the small-scale farmer. Some bioactive compounds including saponins show promise as botanical insecticides and are suited to use in water extracts that are typically used by small holders (Stevenson et al., 2009) while non polar compounds are not. One possible approach for small holders would be the use of soaps in the extraction which can optimize both the extraction efficiency of less polar compounds (Stevenson et al., 2012) but also improve spreading and sticking when applied in field trials (Amoabeng et al., 2013; Mkenda et al., 2015).

3. Insecticidal activity of *A. conyzoides*

A. conyzoides has been shown to have insecticidal activity against several crop pests including cowpea weevil [(*Callosobruchus maculatus* F.) (Coleoptera: Bruchidae)] a destructive pest of cowpea [(*Vigna radiata* L.) (Fabales: Fabaceae)] (Gbolade et al., 1999); the maize weevil [(*Sitophilus zeamais* Motsch.) (Coleoptera: Curculionidae)] (Bouda et al., 2001); the desert locust [(*Schistocerca gregaria* Forskal) (Orthoptera: Acrididae)] (Pari et al., 1998); sorghum stalk borer [(*Chilo partellus* Swinh.) (Lepidoptera: Pyralidae)] (Raja et al., 1987); rice weevils [(*Sitophilus oryza* L.) (Coleoptera: Curculionidae)], a sphingid moth [(*Theretra japonica* Bois.) (Lepidoptera: Sphingidae)], the rice bug [(*Leptocorisa chinensis* Dallas) (Hemiptera: Alydidae)] and the red cotton stainer [(*Disdercus flavidus* Sign.) (Hemiptera: Pyrrhocoridae)] (Fagoonee and Umrit, 1981). The petroleum ether extract at 0.5-1.5 % (w/w) of benzene diluted extract applied to green gram repelled 99% of cowpea weevil (*Callosobruchus chinensis* L.) (Coleoptera: Bruchidae)] over a 10-day exposure period. An admixture of 1.5% (w/w) reduced the weight loss of infested greengram to 0.46% compared to 38% in the untreated control. The benzene control samples also repelled 99% of *C. chinensis* and reduced weight loss to 8% which may indicate that the insecticidal effect was associated with the solvent (Pandey et al., 1986). Similarly, the application of the volatile oil of *A. conyzoides* on cowpea seed exhibited insecticidal activity against *C. chinensis* (Gbolade et al., 1999; Bouda et al., 2001). It has also been indicated that the essential oil of *A. conyzoides* at 5mg/50g mungbean seed caused 97% mortality in adult *C. chinensis* within 24 hours and completely prevented egg-laying (Morsllo-Rejesus et al., 1990). Moreira et al. (2004) reported that the hexane extract from leaves of *A. conyzoides* showed significant insecticidal activity leading to 100% mortality of Melonworm [(*Diaphania hyalinata* L.) (Lepidoptera: Crambidae)] which is a key pest of cucumber after just 4 hours of exposure.

Liang and Huang (1994) reported that intercropping *A. conyzoides* enhanced the numbers of predatory mites (*Amblyseius* spp) (Mesostigmata: Phytoseiidae) in a citrus orchard. This genus of predatory mite is an effective natural enemy of a key Citrus pest; the citrus red mite [(*Panonychus citri* McGregor) (Acari: Tetranychidae)]. Similarly Pu et al. (1990) reported that *A. conyzoides* in citrus orchards provided refuge and shelter for predators of the spider mite (*P. citri*). They further noted a reduction of other spider mite populations; *Phyllocoptruta oleivora* Ashm. (Prostigmata: Eriophyidae) and *Brevipalpus phoenicis* Geijskes (Acarina: Tenuipalpidae) were decreased with maintenance of *A. conyzoides* in the orchards.

Numerous other effects of *A. conyzoides* against insects are reported. The leaf, flower and root extracts of *A. conyzoides* were toxic to the cotton stainer [(*Dysdercus angulatus* Fabricius) (Hemiptera: Pyrrhocoridae)], maize weevil (*S. zeamais*) and red flour beetle [(*Tribolium castaneum* Herbst) (Coleoptera: Tenebrionidae)] (Carino, 1981). Fagoonee and Umrit (1981) reported that a crude lipid extract from this plant showed ovicidal activity and reduced fertility of the cotton stainer (*D. flavidus*) when applied topically on its 5th instar larvae and the adult females. This crude extract was shown to contain precocene I and II. Fagoonee and Umrit (1981) further reported that water extract of leaf and branch of *A. conyzoides* showed insecticidal activity against

D. flavidus while the same water extract showed insecticidal activity on the 5th instar larvae of the potato tuber moth [(*Gnorimoschema operculella* Zeller) (Lepidoptera: Gelechiidae)] (Pandey et al., 1982) and the adults of the Vinegar fly [(*Drosophila melanogaster* Meigen) (Diptera: Drosophilidae)] (Padolina, 1983). Its whole plant powder admixed with stored wheat seed significantly reduced the grain damage and population of the rice weevil (*S. oryzae*) (Rout, 1986).

However, these reports illustrate a major oversight of many studies on the biological activity of plants against pests – a lack of chemistry (Isman and Grieneisen, 2014). Where these activities are reported with chemistry underlying the activity there is much greater scope to exploit the activity and inform how industrial products might be developed. For example, ageratochromene has been isolated from the genus *Ageratum* along with its hydroxyl derivatives and shown to have anti-juvenile hormonal activity on insects (Bowers et al., 1976). Precocenes (ageratochromenes) were toxic to stored beetle [(*Oryzaephilus surinamensis* L.) (Coleoptera: Silvanidae)] (Saleem and Wilkins, 1986) and showed inhibition of juvenile hormone dependent reproduction in Mexican bean beetle [(*Epilachna varivestis* Mulsant) (Coleoptera: Coccinellidae)], induction of adult-diapause in Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae)], (Bowers et al., 1976), flight activity inhibition in Convergent lady beetle (*Hippodania convergen* Guerin) (Coleoptera: Coccinellidae)] (Rankin and Rankin, 1980), juvenilization of 5th instar of Migratory locust [(*Locusta migratoria* L.) (Orthoptera: Acrididae)] (Miall and Mordue, 1980) and prolongation of larval-pupal period of *Spodoptera mauritia* (Boisduvalii) (Lepidoptera: Noctuidae) (Mathai and Nair, 1983). Lu (1982) reported that precocene I and II were highly toxic to the rice weevil (*S. oryzae*) and rice earhead bug (*L. chinesis*). Precocene II was also found to cause morphological abnormalities in the treated pupae of *Epilachna vigintioctopunctata* Fabricius. (Coleoptera: Coccinellidae)], when applied topically

(Gupta and Dogra, 1990). Leaf volatile oils of *A. conyzoides* have been reported to kill the maize grain weevil (*S. zeamais* (Bouda et al., 2001). So these chromenes present a potential commercial opportunity and with a plant that, as a pernicious weed, is likely to be easy to propagate we predict *A. conyzoides* offers a viable new botanical pesticide opportunity.

In studies investigating the potential field applications of botanicals Amoabeng et al. (2013) tested nine different species as botanicals including *A. conyzoides* and *Chromolaena odorata* [(L.) (King and Robinson) (Asterales: Asteraceae)]. This required the collection of fresh leaves from different plants and locations and then taking a sub-sample of 30g fresh weight of each. These were then pounded into a pulp in a wooden mortar using a wooden pestle. The processed materials were each mixed with 1 L tap water containing 0.1% Sunlight® detergent solution to give a 3 % w/v final concentration then sieved through fine linen into a 2 L capacity hand sprayer for immediate application. They reported that while plots treated with extracts of either *A. conyzoides* or *C. odorata* had lower head weight of cabbage, compared to Attack® (conventional insecticide), there was effective control of *Plutella xylostella* (L) (Lepidoptera: Plutellidae) and *Brevicoryne brassicae* (L) (Hemiptera: Aphididae) compared to no treatment but there were also added advantages of using the botanicals. The plant extracts were significantly less harmful to beneficial natural enemies including ladybirds [(*Coccinella magnifica*) (Redtenbacher) (Coleoptera: Coccinellidae)], Hoverflies [(*Episyrphus balteatus*) (De Geer) (Diptera: Syrphidae)] and spiders (Clerck.) (Araneae). Preserving natural enemies may reduce the frequency with which insecticide applications are required since they can attack and kill pests that survive the application of botanicals. Furthermore, Amoabeng et al. (2014) reported that using crude extracts of botanicals including *A. conyzoides* was as economical as synthetics and more relevant to resource limited farmers who have adequate labour to prepare the botanicals but perhaps not the resources to invest in the commercial products. The economic benefits would be enhanced significantly for entrepreneurial farmers who chose to grow more plant material than required for personal use and sell locally but upscaling to an industrial level may also be viable for this species.

In efforts to upscale the use of *A. conyzoides* more plant material would be required to sustain the production and marketing of the new botanical pesticide. This plant has inherent characteristics that will contribute to the efforts of supply of raw materials for the same. This plant can grow from sea level to a very high altitude of upto 2400 m above sea level (Wagner et al., 1999; Dogra et al., 2009) suggesting that it grows almost in all areas and therefore can be suitable for cultivation in many places. It has a high potential rate of seed production (94,772 seeds per plant) with a 5-8 months period of seed shedding and upto half of seed germinating (Holm et al., 1977), resulting in the abundance emergence of plants (1000 plants m^{-2}) (Ekeleme et al. 2005). Moreover, its extraordinary physiological plasticity has enhanced its persistence in agricultural fields (Ekeleme et al. 2005). This therefore presents this crop with great potential to supply adequate and cheap source of raw materials whether cultivated or in-situ to sustain biopesticide product development by small-scale farmers.

Botanicals may be an alternative when synthetics are no longer effective owing to the development of resistance. For example, Kumar et al. (2016) in their studies on the acaricidal effects of *A. conyzoides* reported that ethanolic extract caused a LC90 value of 8.91% against reference susceptible IVRI-1 line of *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini) (Acari: Ixodidae). The ethanolic extract was found efficacious against 76.7-90% acaricide-resistant field ticks and adversely affected oviposition showing 7.04-31.3% reduction in egg laying capacity. The extract also showed an in vitro efficacy of 52.5 and 76.7 % against reference resistant IVRI-4 and 5 lines.

Overall, extracts obtained from *A. conyzoides* have been shown to exhibit a variety of mechanisms of activity for the control of insect pests. These effects have been attributed primarily to the hormonal action of precocene I and II. This activity is expressed through the reduction in larval emergence of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949 (Tylenchida: Heteroderidae) (Shabana et al., 1990), accelerated larval morphogenesis resulting into juvenile forms or weak and small adults (Vyas and Mulchandani, 1980), production of sterile and dying adults (Ekundayo et al., 1988), juvenile hormone deficiency (Raja et al., 1987) resulting into morphogenetic abnormalities such as discoloured and longer pupae and incompletely developed adults (Sujatha et al., 1988) and unmelanised and inhibition of development in larval stage and adults with deformed wings, loss of fecundity, lower egg production and production of defensive eggs (Saxena et al., 1992).

4. Antimicrobial effects of *A. conyzoides*

Much of the focus for botanicals in agriculture is on their insecticidal activity (Isman and Grieneisen, 2014) but other pests and diseases may also be susceptible to their biological effects. Plant- based fungicides like those that have activity against insects described above may also be economically viable but importantly locally available to more remote farmers as well as being environmentally benign (Singh et al., 1986; Dubey, 1991; Alam et al., 2002).

For example, acetone extracts of *A. conyzoides* showed antifungal activity against the wilt causing fungus (*Fusarium oxysporum*) (Schltdl.) (Hypocreales: Nectriaceae) indicating that *A.*

conyzoides could be developed also for fungicidal formulations which are environmentally benign and cost-effective (Pal et al., 2013; Srivastava and Singh, 2011). Under *A. conyzoides*-citrus intercropped orchards (Kong et al., 2004c) populations of major pathogenic fungi such as *Phytophthora citrophthora* [(R.E. Sm. & E.H. Sm.) (Leonian) (Pythiales: Pythiaceae)] *Pythium aphanidermatum* [(Edson) (Fitzp.)] (Peronosporales: Pythiaceae)] and *Fusarium solani* (Mart) (Sacc.) (Hypochreales: Nectriaceae) were reduced because Ageratochroromene and its phenolic acids significantly inhibited the spore germination of soil pathogenic fungi although they noted that the two dimers of ageratochromene had no inhibitory action on them. Pal et al. (2013) studied the antifungal activity of some common weed extracts against seed-borne phytotopathogenic fungi *Alternaria* spp. They reported antifungal activity in extracts of *A. conyzoides* and *Parthenium hysterophorus* (L.) (Asterales: Asteraceae) against *Alternaria* spp (Moniliales: Dematiacea)

The mechanisms of effect of antifungal activity have been investigated. For example, precocene II has been found to inhibit trichothecene production by the fungus *F. graminearum* without inhibiting fungal growth (Yaguchi et al., 2009). *F. graminearum* is the predominat plant pathogen in Fusarium Head Blight and produces trichothecene mycotoxins such as deoxynivalenol in infected grains (Pestka and Smolinski, 2005). Chen et al. (2007) indicated that specific inhibitors of trichothecene production such as precocene II are useful for controlling trichothecene contamination without incurring the rapid spread of resultant strains. Kong (2006) showed that ageratochromene and flavones could significantly inhibit spore germination of the *P. citrophthora*, *P. aphanidermatum* and *F. solani* but two dimers of ageratochromene had no inhibitory effects on them. So based on their activity against insects and fungi there is scope to consider products based on *Ageratum* chromenes as potential multiple use botanicals but specific knowledge about the activities of each compounds would be required to optimize activity.

The essential oil of *A. conyzoides* was found to inhibit the growth and production of toxigenic strain of *Aspergillus parasiticus* (Spear) (Eurotiales: Trichocomaceae) (Patil et al., 2010), a new biological activity which indicates a useful tool for a better understanding of the complex pathway of aflatoxin biosynthesis (Nogueira et al., 2010). In their study on the antioxidant, antiaflatoxigenic and antimicrobial activities of *A. conyzoides*, Patil et al. (2010) concluded that the essential oil of *A. conyzoides* could be considered as an alternative natural fungicide for stored products on the basis that its essential oil showed inhibition of aflatoxin production. They noted that the essential oil (0.75mg mL^{-1}) inhibited the growth of *A. parasiticus* and inhibited more than 84% aflatoxin production of the test fungi at a concentration of 0.5 mg mL^{-1}. This biological activity was linked to its antioxidant activity so presents *A. conyzoides* as a potential plant for stored product protection and management of aflatoxin contamination in food and feed using its essential oil as well as macerated green leaf tissue as fumigants.

Javed and Bashir (2012) evaluated the antifungal activity of different extracts of *A. conyzoides* for the management of *F. solani* in Pakistan and reported that concentrations of 6% of the plant extracts of aerial parts of *A. conyzoides* significantly reduced biomass production of *F. solani* indicating its fungitoxic potential worth exploiting for biological management of plant diseases caused by pathogenic fungi.

Fiori et al. (2000) studied the antifungal activity of leaf extracts and essential oils of *Achillea millefolium* (L.) (Asterales: Asteraceae), *Cymbopogon citratus* [(DC) (Stapf)] (Poales: Poaceae), *Eucalyptus citriodora* [(Hook.) (Hill and Johnson)] (Myrtales: Myrtaceae) and *A. conyzoides* on *Didymella bryoniae* [(Fucke) (Rehm) (Pleosporales: Didymellaceae)] (the pathogenic fungi that causes Gummy Stem Blight; considered as one of the most important diseases affecting melon causing damage to leaves and fruits). Their results revealed that crude extracts of *E. citriodora* and *A. conyzoides* were more effective in inhibiting the mycelial growth of *D. bryoniae* whereas *A. conyzoides* was responsible for 52% spore germination inhibition. Among the tested plant extracts, *A. conyzoides* provided 100% inhibition in the mycelial growth and germination of spores of *D. bryoniae*. Iqbal et al. (2001) tested *A. conyzoides* among other weed species against *Aspergillus niger* (van Tieghem) (Eurotiales: Trichocomaceae) which causes fruit rot in tomato, *Rhizoctonia solani* (Kuhn) (Cantharellales: Ceratobasidiaceae), *Lasiodiplodia* (*Botryodiploidia*) *theobromae* (Pat.) Griffon & Maubl. (Botryosphaeriale: Botryosphaeriaceae) and *Pestalotiopsis theae* [(Sawada) (Steyaert)] (Xylariales: Amphisphaeriaceae). They observed that of the five weed species tested, *A. conyzoides* was the most effective against the *R. solani*, *A. niger* and *P. theae*. It inhibited the growth of mycelium by at least 70%. This antifungal activity was maintained up to 9 days.

Several fungal pathogens such as *R. solani*, *Bortrytis cinerea* (Pers.) (Helotiales: Sclerotiniaceae) and *Sclerotinia sclerotiorum* [(Lib.) (de Bary)] (Helotiales: Sclerotiniaceae)]were significantly inhibited by the essential oils of *A.conyzoides* (Kong et al., 2001; 2002a). *A. conyzoides* has also been shown to be a symptomless carrier of *Burkholderia solanacearum* (Smith) (Burkholderiales: Ralstoniaceae) which is an important pathogen in potato in India (Sunaina et al., 1989).

5. Herbicidal effects of *A. conyzoides*

Ageratum conyzoides is itself a weed. In fact, it is among the weeds listed as the most economically destructive in the world (Abul-Fatih and Bazzaz, 1979; Holms, 1991; Ballard et al. 1996; Ghisalberti, 2000; Okunade, 2002) and this may be because of its allelopathy against other plants. Allelopathic weeds contain numerous plant-growth inhibitors that could be used for the development of natural herbicides (Duke et al. 2000; Vyvyan, 2002; Macias et al., 2007). So, ironically, there may be scope to use *A. conyzoides* to control other weed species. Most allelopathic weeds are economically destructive to crop production and as with fungi perhaps overlooked as potential targets in the development of botanical crop protection applications. However, some allelopathic weeds and their allelochemicals can be used for pest management and control in agricultural ecosystems or employed for biorational pesticides (Duke et al., 2000; Kong et al., 2006; Macias et al., 2007) and the potential use of allelopathic weeds as an agricultural resource was also recognized by Ming (1999). Where specific activities can be attributed to specific chemicals their development in to biorational pesticides is possible and even through synthesizing the bioactive compounds (Duke et al., 2000; Vyvyan, 2002; Macias et al., 2007; Tabaglio et al., 2008). Such an allelopathy-based weed management approach is being developed for sustainable agriculture in the low-input crop-farming systems that are prevalent throughout China and other Asian countries (Kathiresan, 2000; Kong et al., 2004c; Xuan et al., 2005).

Kong et al. (2004c, 2005, 2007) conducted studies in China on allelopathic weeds including *A. conyzoides* and showed that the allelopathic properties and allelochemicals of these weeds have been used for ecological pest management and control by incorporation into herbicides.

The use of allelopathic plant mulches for ecological pest management and control has received a attention (Everall and Less, 1997; Hong et al., 2004). These weeds may be used as covering chips or intercropping species for pathogen and weed reduction (Kong et al., 2004c; Xuan et al., 2005).

The mulches of several allelopathic weeds including *A. conyzoides* might be useful as alternative materials for biological weed control and particularly for the reduction of herbicide use in paddy fields. These allelopathic weeds promoted rice growth and yield and greatly reduced paddy weed growth at a dose of 20 tons/ha (Hong et al., 2004; Khanh et al., 2005). Herbicidal effect in paddy fields have been reported for *A. conyzoides* (Xuan et al., 2004) where application of 2 ton/ha served as an effective herbicide in controlling paddy weeds such as *Echinochloa crus-galli* [(L.) (Beauv.)] var. *formosensis* (Ohwi) (Poales: Poaceae), *Monochoria vaginalis* [(Burm. F.) (Presl)] var. *plantaginea* [(Roxb.) (Solms-Laubat)] (Commelinales: Pontederiaceae) and *Aeschynomene indica*.

A. conyzoides is highly adaptable and spreads vegetatively through stolons (Okunade, 2002). It becomes a destructive weed of arable land interfering with the growth and development of crop plants (Kohli et al., 2006). It is resistant to insects and diseases which may be explained by its rich biologically active chemistry. Through leaching, volatilizing and residue decomposition, *A. conyzoides* releases allelochemicals into the environment to exert an allelopathic effect on other plants. Its allelopathic potential varies with its growth stages and environmental factors (Kong et al., 2002a, 2004c). Under favourable conditions, *A. conyzoides* is less allelopathic, but becomes more allelopathic under adverse conditions particularly under nutrient deficiencies and competition with other plant species so may be well suited to poor quality agricultural land. It is also more allelopathic after infection with pathogen *Erysiphe cichoracearum* (DC) (Erysiphales: Erysiphaceae) and attack by the pest, *Aphis gossypii* (Glover)

(Hemiptera: Aphididae) (Kong et al., 2002a). So, while the species provides a potential opportunity for commercial or homegrown botanicals care must be taken by growers because of its ability to invade disturbed environments, particularly agricultural land.

6 Nematicidal effects and miscellaneous uses of *A. Conyzoides*

Some pesticidal plants provide a reservoir for nematodes. *Tephrosia* (Pers.) (Fabales: Fabaceae) species for example are well known for their pesticidal activity (Stevenson et al., 2012; Belmain et al., 2012; Mkenda et al., 2015) but also harbor nematodes as reported for *Tephrosia candida* (Throwler, 1958). Similarly, *A. conyzoides* has been reported to be a host of the banana nematodes (*Radopholus similis* (Thorne) (Tylenchida: Pratylenchidae) and *Helicotylenchus multicinctus* (Gold.) (Tylenchida: Hoplolaimidae) in Brazil (Zem, 1983) and of the root knot nematode (*Meloidogyne javanica*) (Treub) (Tylenchida: Heteroderidae) (Mamaril and Alberto, 1989) so care must be taken when considering planting this species on large scale in case it exacerbates nematode populations. Conversely, Wabo et al. (2011c) reported that aqueous and ethanolic extracts of *A. conyzoides* showed ovicidal and larvicidal properties against the parasitic nematode *Heligmosomoides bakeri* (Cable) (Nematoda: Heligmosomatidae).

Here we argue that *A. conyzoides* provides a useful and potentially commercial resource for control of agricultural pests and diseases. This potential could be augmented, however, by some additional uses that enhance agricultural environments. For example, *A. conyzoides* planted as a riparian wetland herb on the banks of the Rihand River in Renukoot reduced erosion of organic carbon and cationic nutrients and helps in soil conservation (Kumar et al., 1996). *A. conyzoides* also maintains the texture and fertility of soil along rivers through reducing soil erosion (Srivastava et al., 2009). *A. conyzoides* was even reported to be used successfully as a substrate for oyster mushroom cultivation that helps to increase its protein content and production time (Nirmalendu and Mina, 2007). Finally, in South China *A. conyzoides* is used as green manure in fields to increase the crop yields (Liang and Huang, 1994; Kong et al., 2004b).

7. Recommended further studies for *A. conyzoides*.

1. *A. conyzoides* contains precocene I and II which have been shown to cause hepatotoxicity thus further research is needed to understand what risk there is to consumers and users when applied to stored grains for human consumption or field crops.
2. Insufficient research has evaluated the effects of *A. conyzoides* extracts on field pests or how best to formulate and apply the extracts. Some recent evidence suggests soap extracts optimize activity and improve sticking and spreading which may suit small holder farming (Belmain, 2012 and Mkenda, 2015).
3. Isolation of the active antifungal compounds followed by testing them against other pathogenic fungi for control of other different diseases would help to determine the components that are responsible for the effects and identify potential targets for optimizing production.
4. The findings by Kong et al. (2004), indicated that the level of allelochemicals and hence allelopathic potential depends upon the growth stage and type of habitat. However, little is known about what stage of growth *A. conyzoides* exhibits maximum allelopathic potential on weeds, fungi or insects.
5. Josep and Joan (1997), Kong et al., (2002) and Batish et al. (2009a & b) indicated that allelochemicals may have synergistic effects, and their allelopathic potential is intensified on

exposure to various environmental stresses. These findings have not yet been verified through research for *A. conyzoides* against various weeds but could help optimize use.

6. *A. conyzoides* has been shown to exhibit herbicidal effects in paddy fields but little work has been conducted on other crop systems.
7. Insufficient information is known about the correct application rates of plant extracts of the *A. conyzoides* as a bioherbicide but this would be improved by knowing more about the chemistry of the activity.

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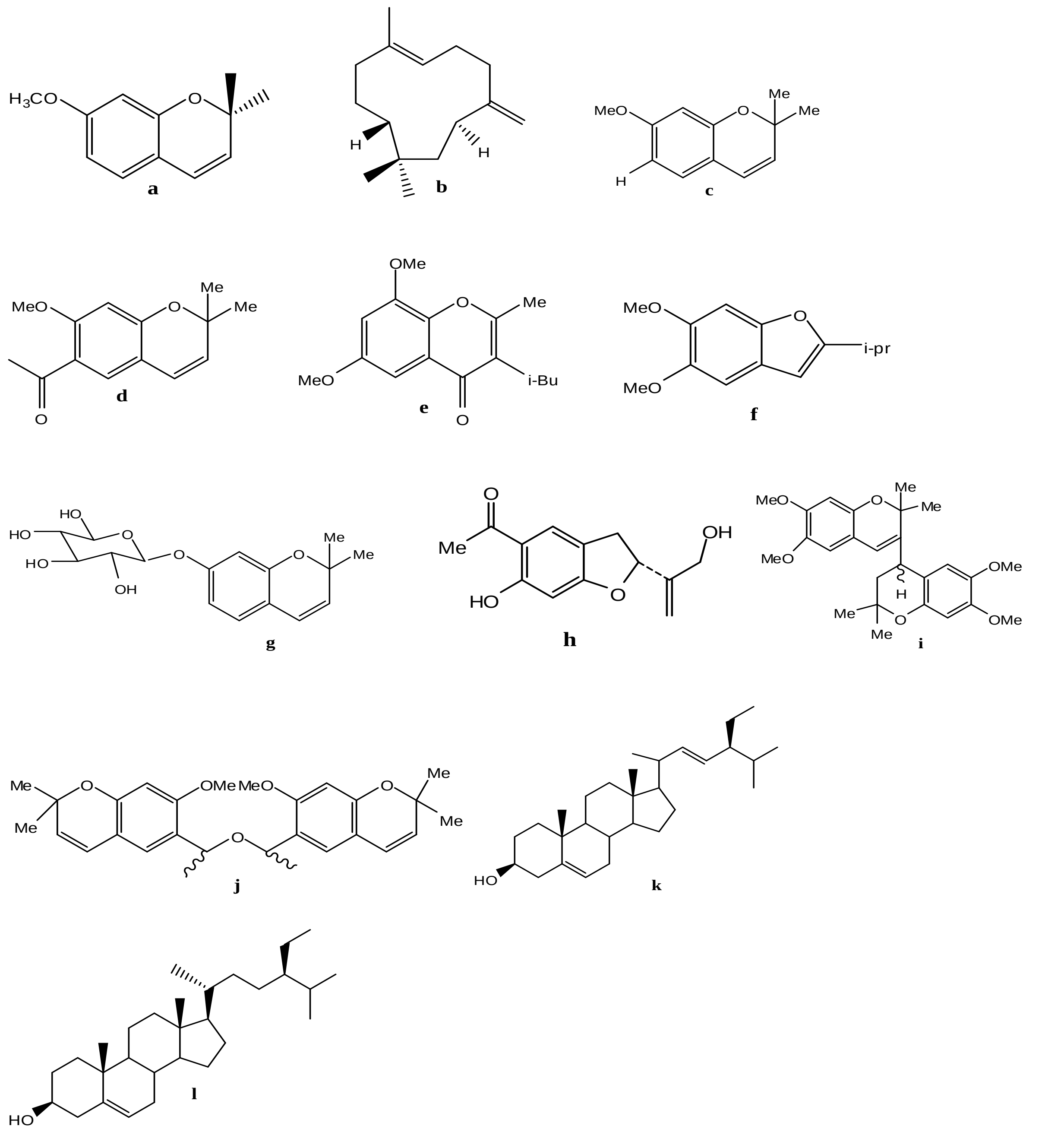

Figure 1. Chemical structures of: **a**. precocene I, **b.** (*E*)-caryophyllene, **c.** precene I; **d.** procene II, **e.** 3-(2'-methylpropyl)-2-methyl-6,8-demethoxychrom-4-one, f. 2-(2'-methylethyl)-5,6-dimethoxybenzofuran, **g.** 2,2-dimethylchromene-7-*O*-β-glucopyranoside **h.** 14-Hydroxy-2H beta3-dihydroeuparine, **i**. ageratochromene dimer, **j.** encecanescin, **k.** stigmasterol, **l.** β-sitosterol