



Allelochemicals from *Parthenium* for water hyacinth control

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ABSTRACT

Water hyacinth is an aquatic plant coined to multifarious activities including its role as an obnoxious weed with tremendous economic and aesthetic implications. *Parthenium* (*Parthenium hysterophorus* L.) is a terrestrial weed often put in the category of world's worst weeds now assuming status of India's national weed affecting human health, agriculture, environment and natural biodiversity with tremendous economic implications. The weed has toxic and phytotoxic constituents comprising of phenolics and terpenoids- two major chemical classes implicated in toxic and allelopathic interactions of the species. The species has wide range of constituents in its plant parts, but a few allelochemicals have been investigated for water hyacinth control. Many phenolic acids have been investigated for inhibitory activity on water hyacinth, of which *p*-hydroxybenzoic acid appeared to be of potential herbicidal activity at 100 ppm, a lethal level for the aquatic weed. Major sesquiterpene lactone parthenin is another allelochemical which has been shown to be a potential herbicidal for water hyacinth at 100 ppm, which killed the weed irrecoverably. Investigations undertaken on the aspect of control of water hyacinth by *Parthenium* allelochemicals showed that allelochemical crude and constituent allelochemicals and other secondary metabolites from *Parthenium* leaf herbicidal for the water hyacinth. The allelochemicals could be used under certain situations for managing a weed (water hyacinth) by another weed (*Parthenium*) fostering a concept of weed against weed. The little work undertaken so far on screening of *Parthenium* constituents including allelochemicals for herbicidal activity on water hyacinth and other aquatic weeds pointer to necessity of taking up the investigations on these lines intensively, which might facilitate the development of natural herbicides and their formulations and provide lead for the development of newer synthetic herbicides with novel chemistry for more effective and environment friendly management of water hyacinth and other aquatic weeds.

Key words: Allelochemicals, Management, Parthenin, *Parthenium*, Phenolics, Water hyacinth

Water hyacinth (*Eichhornia crassipes* Mart. Solms) is one of the worst aquatic plants in the world. It is native to South America, but has been naturalized in most of the parts of the world's subtropical and tropical climates. Water hyacinth plants have tremendous growth and reproductive rates and the free-floating mats cause substantial problems. Plant managers and water front residents spend millions of dollars per year for its management. The plant reproduces by seeds and vegetatively through daughter plants that form on rhizomes and produce dense plant beds. A single plant can produce as many as 5,000 seeds and waterfowl eat and transport seeds to new locations. The best way to manage water hyacinth is to prevent it from ever becoming established (Anonymous 2014). Water hyacinth reproduces sexually by seeds and vegetatively by budding and stolon production. Daughter plants sprout from the stolons and doubling times have been reported of 6-18 days. The seeds can germinate in a

few days or remain dormant for 15-20 years. They usually sink and remain dormant until periods of stress (droughts). Westerdahl and Getsinge (1988) reported excellent control of water hyacinth by the use of the aquatic herbicides 2,4-D or diquat. Mechanical controls such as harvesting have been used for nearly 100 years in Florida, but are ineffective for large scale control, very expensive, and cannot keep pace with the rapid plant growth in large water systems. Three insects have been released for the biological control (Grodowitz 1998) of water hyacinth. These include two weevil species (*Neochetina* spp.) and a moth (*Sameodes albittalis*). Unfortunately large scale reductions in water hyacinth populations did not occur.

Parthenium hysterophorus L. is commonly known as American feverfew, white top, white head, carrot grass (for resemblance of its leaves with that of carrot) or congress-grass. The species is a herbaceous annual or ephemeral of Asteraceae with world-wide occurrence and is a major crop and

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pasture weed of India and Australia in particular (Towers *et al.* 1977, Navie *et al.* 1996, Pandey *et al.* 2003, Sushilkumar and Varshney 2007). It has been asserted that photo- and thermo-insensitivity and adaptability to contrasting ecological conditions enable it to thrive from sea level to altitudes of 3000 m above mean sea level (amsl) (Lomniczi de Upton *et al.* 1999) in Argentina and to altitudes of more than 2080 m amsl in the Central Himalaya in India (Pandey *et al.* 2003). In the absence of effective natural enemy, its much publicized than verified probable allelopathic effects on other species and rapid growth allow it to grow luxuriantly all through the year, except in extreme winter, especially with spells of freezing temperatures, and summer drought, suppressing native vegetation and threatening biodiversity (Krishnamurthy *et al.* 1977, Towers *et al.* 1977, Kanchan and Jayachandra 1979a, b, 1980a, b, c, Williams and Groves 1980, Jayanth and Bali 1994, Pandey *et al.* 2003). This may threaten quality and quantity of agricultural production, human and animal health, biodiversity and the environment resulting in serious socio-economic implications (Towers *et al.* 1977, Narasimhan *et al.* 1977, Pandey *et al.* 2003). The biological interactions of the species are due to its allelochemicals comprised of phenolics and terpenoids including sesquiterpene lactone parthenin as a major constituent (Rodriguez *et al.* 1971, Picman *et al.* 1979, Picman *et al.* 1980, Kanchan and Jayachandra 1980b, Picman *et al.* 1981, Das and Das 1995). Economic potential of the secondary metabolites including allelochemical constituents remains to be speculative, and descriptive rather than drawing practical advantage for use in agriculture and agricultural pest management.

Allelochemicals in *Parthenium*

Investigations on natural product chemistry of *Parthenium* have been confined to major nutrients (Dutta *et al.* 1979), phenolics, organic acids, sesquiterpene lactones (Rodriguez *et al.* 1971, Rodriguez *et al.* 1976, Picman *et al.* 1979, Picman *et al.* 1980, Kanchan and Jayachandra 1980b, Picman *et al.* 1981, Das and Das 1995) and leaf oils (Kumamoto *et al.* 1985) (Table 1 and Table 2).

Though the *Parthenium* plant has a vast array of secondary metabolites, yet nature of most of the constituents as to being allelochemicals is to be ascertained. It is likely that many yet to be investigated constituents might show allelopathic potential of varying degrees and many may have high herbicidal activity to control water hyacinth. Phenolic acids identified from *Parthenium* plant parts with

reference to allelochemic-interactions include caffeic acid, vanilic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid and *p*-hydroxybenzoic acid and among the organic acids, fumaric acid (Kanchan and Jayachandra 1980b, Das and Das 1995). Among pseudoguaianolides reported are parthenin, coronopilin, damsin, hymanin, 8-b-hydroxyparthenin, anhydroparthenin, hysterin, tetraeurin, ambrosanolides and *p*-methoxybenzoic acid, a sterol β -sitosterol, a triterpenoid betulin, flavonoides quercetagenin-3,7-dimethyl ether, 6-hydroxykempferol 3, 7-dimethyl ether, kaempferol 3-0-glucoside, quercetin 3-0 glucoside and kaempferol-3-0-glucoarabinoside, and a rare lignan (+)-syringaresinol (Rodriguez *et al.* 1971, Rodriguez *et al.* 1976, Picman *et al.* 1980, Towers *et al.* 1977, Kanchan and Jayachandra 1980b, Das and Das 1995, Lomniczi de Upton *et al.* 1999, Venkataiah *et al.* 2003). Occurrence and concentration of various constituents depended on plant parts, geographical distribution and specific populations (Towers *et al.* 1977, Lomniczi de Upton *et al.* 1999). Fresh leaves of *Parthenium* yielded about 0.033% oil which comprised of μ -pinene, camphene, β -pinene, sabinene, β -myrcene, α -terpinene, limolene, β -ocimene, ocimene, *p*-cymene, linalool, caryophyllene, humulene, terpinene-4-ol and many unidentified components (Kumamoto *et al.* 1985). Parthenin and related sesquiterpene lactones form adducts with cysteine and glutathione (Picman *et al.* 1979). The plant and its residue owe a range of biological activities to constituent phenolics and major sesquiterpene lactone parthenin.

Parthenium plant parts herbicidal to water hyacinth

Initial exploratory studies showed that *Parthenium* plant was inhibitory to water hyacinth. Investigations were undertaken to study inhibitory activity of different plant parts of the *Parthenium* on this and other floating and submerged aquatic weeds. Experimental results revealed that residue of *Parthenium* plant and its parts were herbicidal to water hyacinth at 0.1-2.0% (dry w/v) (Pandey *et al.* 1993a, b). The herbicidal activity of the plant and plant parts residue appeared to be due to allelochemicals comprising phenolics and terpenoids, major groups of secondary metabolites implicated in its allelopathy (Kanchan and Jayachandra 1979a).

Effect of *Parthenium* leaf residue and its allelochemicals: *Parthenium* dry leaf powder (DLP) was inhibitory to water hyacinth (Pandey *et al.* 1993a). The DLP caused wilting starting from the margins of the older leaves and desiccation of above-

Table 1. Phenolics and alkaloid secondary metabolites (including allelochemicals) in *Parthenium* and their reported biological activities

Constituent	Reference	Reported biological activity of the constituent	Reference
<i>Phenolic acids</i>			
Occurrence: leaf, stem, root, flower, pollen and trichomes, depending on plant parts and other factors. Usual cellular localization is in vacuoles.			
1. Caffeic acid	Kanchan and Jayachandra (1980b), Das and Das (1995)	allelopathy, phytotoxicity, herbicidal activity, growth regulation / inhibition; and nitrification and nitrifying bacteria	Gross 1975, Lodhi and Killingbeck 1980, Patterson 1981, Rice 1984, Mersie and Singh 1988, Pandey 1994b, Pandey 1996b, Pandey and Mishra, 2002, Pandey and Mishra 2005
2. Vanillic acid			
3. Ferulic acid			
4. Chlorogenic acid			
5. <i>p</i> -Coumaric acid			
6. <i>p</i> -Hydroxy benzoic acid			
<i>Flavonoids</i>			
Occurrence: leaf, stem, flower and pollen, depending on plant parts and other factors. Usual cellular localization is in vacuoles.			
1. Quercetageitin-3,7-dimethyl ether	Rodriguez <i>et al.</i> (1971), Rodriguez (1977), Towers <i>et al.</i> (1977)	antioxidant activities, scavenging effects on activated carcinogens and mutagens, action on cell cycle progression, altered gene expression, UV-B protection in plants, warding off microbial infections, and protection of plants from herbivores, etc.	Harborne and Williams 2000, Rusak <i>et al.</i> (2002)
2. 6-Hydroxy kempferol-3,7-dimethyl ether			
3. Kaempferol 3-O-glucoside			
4. Quercetin 3-O-glucoside			
5. Kaempferol-3-O-glucoarabinoside			
6. Lignan (+) - syringaresinol			
<i>Alkaloids</i>			
Occurrence: leaf, stem, root, flower. Usual cellular localization is in vacuoles.			
Alkaloids-2 (Unidentified)	Rodriguez 1977	-	-

water plant parts (shoot). Appearance, persistence, and disappearance of symptoms depended on the level and duration of the treatment and recovery of the treated plants, if it occurred. The treatment drastically reduced the number of healthy leaves (HLN) and the plant biomass at 0.25% (w/v) DLP; the treated plants recovered in about one month. At and above 0.50% (w/v) DLP, the plants were killed in about one month, resulting in sinking of the dead mass in water. The results indicated that the inhibitors leached out of the DLP affected the water hyacinth plants through changes in macromolecules: protein, lipid, and nucleic acid, resulting in root dysfunction and other inhibitory activities both in the root and shoot. Phenolic and other inhibitors including those found in the *Parthenium* plant (except sesquiterpene lactones which had not been tested) at 50 ppm, except *p*-hydroxybenzoic acid, did not affect the treated plants. Such a high concentration of the allelochemicals was unlikely to be present in the medium at the lethal dose (0.50% w/v) of the DLP. Even with *p*-hydroxybenzoic acid, the plants recovered subsequently and grew normally. Thus, it appeared that other allelochemicals including sesquiterpene lactones were mainly responsible for the inhibitory activity of the DLP on water hyacinth plants.

Relative effect of flower, leaf, stem, and root residue allelochemicals

Since other plant parts of *Parthenium* also showed inhibitory effects on water hyacinth, relative effect of residue of leaf, flower, stem, and root of *Parthenium* on growth of water hyacinth was studied. The inhibitory activity of the residue as shown by its effect on biomass and healthy leaf number (HLN) of treated plants was in the order: leaf and flower > stem > root (Pandey *et al.* 1993b). Total phenolic acids in the medium after 72 hr of suspending the plant part residue were maximum in flower followed by leaf, root, and stem, successively. The DLP and dry flower powder (DFP) at and above 0.50% (w/v) and dry stem powder (DSP) at 1.0% (w/v) killed water hyacinth in about one month. Dry root powder (DRP) at the highest dose (1.25% w/v) reduced the growth of the treated plants drastically, but the plants recovered after about one month. The DSP at 0.50% (w/v) and DRP at 0.25–0.75% (w/v) supported growth of treated plants, probably due to lower levels of inhibitors, allowing utilization of constituents of the residue as nutrients. Using wheat seedling as a reference material, it was observed that in aquaculture at different levels of *Parthenium* plant parts residue, water hyacinth plants were much more sensitive to inhibitory activity. Thus, water hyacinth

Table 2. Pseudoguaianolide and oils (including allelochemicals) in *Parthenium* and their reported biological activities

Constituent	Reference	Reported biological activity of the constituent	Reference
Occurring in shoots (mainly leaves and flowering heads), trichomes and seedlings in their first true leaves which bear trichomes, depending on plant parts and other factors. Located in the cytoplasm of the plant cells.			
<i>Pseudoguaianolides</i>			
1. Parthenin	Herz and Hogenauer 1961,	Cytotoxic, antitumor, antibacterial, antifungal, phytotoxic, anti-protozoan, activity against human and animal parasites (including intermediate hosts), insecticidal, molluscicidal, vertebrate feeding deterrence and toxicity, allergic contact dermatitis, mitochondrial oxidative phosphorylation inhibition, allelopathic, anti-inflammatory, and antimalarial	Fay and Duke (1977), Narasimhan <i>et al.</i> (1985), Picman (1986), Pandey (1996b), Warsaw and Zug (1996), Sharma and Bhutani (1988), Hooper <i>et al.</i> (1990), Tefera (2002), Verma <i>et al.</i> (2002), Ramesh <i>et al.</i> (2003), Verma <i>et al.</i> (2004), Sharma <i>et al.</i> (2005), Lakshmi and Srinivas (2007), Regina <i>et al.</i> (2007), Das <i>et al.</i> (2007), Krenn <i>et al.</i> (2009)
2. Anhydroparthenin	Romo de Vivar <i>et al.</i> 1966,		
3. Ambrosin	Rodriguez <i>et al.</i> 1976, Towers		
4. Coronopilin	<i>et al.</i> 1977, Picman <i>et al.</i> 1980,		
5. Damsin	Picman <i>et al.</i> 1982, Picman		
6. Hymanin	1986, Venkataiah <i>et al.</i> 2003,		
7. 8- β -Hydroxyparthenin	Ramesh <i>et al.</i> 2003, Das <i>et al.</i>		
8. 2 β -Hydroxycoronopilin	2005, Das <i>et al.</i> 2006, Das <i>et</i>		
9. Tetraneurin-A	<i>al.</i> (2007)		
10. Ambrosanolides			
11. Charminarone			
12. 8- β -Acetoxysterone C			
13. Deacetyltetraneurin A			
14. Hysterin			
15. Hysterone E			
16. Hysterone D			
17. Conchasin A			
18. Acetylated pseudoguaianolides			
19. Scopoletin (belongs to coumarin)			
20. Dihydroxyparthenin			
<i>Oils</i>			
1. α -Pinene	Kumamoto <i>et al.</i> (1985)	Antifungal, antibacterial, antimicrobial, virucidal, antiparasitical, insecticidal, medicinal and with cosmetic applications; and cytotoxic	Uribe <i>et al.</i> (1985), Lima <i>et al.</i> (1993), Velickovic <i>et al.</i> (2002), Damjanovic - Vratnica <i>et al.</i> (2008), Bakkaliet <i>al.</i> (2008), Ogendero <i>et al.</i> (2008)
2. Camphene			
3. β -Pinene			
4. Sabinene			
5. β -Myrcene			
6. α -Terpene			
7. Limolene			
8. β -Ocimene			
9. Ocimene			
10. p-Cymene			
11. Linalool			
12. Caryophyllene			
13. Humulene			
14. Terpinene-4-ol			
15. Many unidentified compounds			

was suggested as a material for bioassay of inhibitory activity of the *Parthenium* plant residue. Simultaneously, the results implicated occurrence of potential herbicidal activity in *Parthenium* plant parts residues tempting exploration of the materials for search for natural molecules with potential herbicidal activity. However, probably tedious efforts and resources required for isolation and characterization of the allelochemicals and secondary metabolites, systematic studies are still lacking on screening of individual constituents of *Parthenium* plant parts for their herbicidal activity.

Allelochemical crude herbicidal to water hyacinth

Allelochemical crude prepared by suspending the *Parthenium* plant parts residue in water and evaporating to dryness showed potential herbicidal activity on water hyacinth. Some of the plant parts had much higher herbicidal potential than others. For instance, inflorescence and leaf allelochemical had higher herbicidal activity than stem and root allelochemical crude (Pandey, unpublished work).

In general the allelochemical crude had a yield of about one fourth to one fifth of dry residue of the

plant parts. The allelochemicals could be further concentrated by using appropriate solvents and the isolated constituents in final purification can be potential herbicidal to water hyacinth inhibiting and killing it at as low as 12-25 ppm under certain outdoor environmental conditions (Pandey, unpublished work). The concentration of allelochemicals to potential herbicidal formulation is being intensively investigated in the laboratory of the author.

Allelochemicals and their inhibitory activity status to water hyacinth

Phenolics (caffeic acid, vanilic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid and *p*-hydroxybenzoic acid) and organic acids (fumaric acid) identified from *Parthenium* plant parts with reference to allelochemic interactions (Kanchan and Jayachandra 1980b, Das and Das 1995) were investigated for their toxicity on water hyacinth (Pandey 1996a). Caffeic acid stopped biomass increment in water hyacinth plants at 100 ppm but showed little or no effect at 25 and 50 ppm. Vanillic acid had inhibited water hyacinth growth at 100 ppm but was not effective inhibitor at 25 and 50 ppm. Ferulic acid reduced biomass growth over the initial values at 100 ppm and reduced the growth over controls at 50 ppm but was not inhibitory at 25 ppm. Chlorogenic acid was inhibitory at 100 ppm but was obviously not inhibitory at 25 and 50 ppm. *p*-Coumaric acid was inhibitory at 100 ppm but was not so at 20 or 50 ppm. *p*-Hydroxybenzoic acid was lethal at 100 ppm, considerably inhibitory at 50 ppm and was not inhibitory at 25 ppm. Fumaric acid was inhibitory at 100 ppm but was not so at 25 and 50 ppm.

The sesquiterpene lactone parthenin, one of the major secondary metabolites and often considered as an allelochemical of *Parthenium* plant, has been shown to be potential phytotoxic to water hyacinth (Pandey 1996b). Parthenin killed water hyacinth at 100 ppm. The concentration just below the lethal level reduced biomass of the plants. Higher concentrations of parthenin killed the treated plants quicker. At lethal level, the treated plants were killed in about 10 days. Parthenin reduced water use by water hyacinth plants. This was apparent as early as two days after initiation of the treatment. The reduction in water use in treated plans was drastic by six to seven days. The massive increase in solute leakage from the roots of water hyacinth plants grown at the lethal dose of parthenin for two, four, and eight days showed damage to cellular membranes that resulted in loss of cellular structures and constituents. Total

dehydrogenase activity in the roots of water hyacinth allowed to grow in 100 ppm parthenin (lethal dose) solution declined rapidly. Enzyme activity declined by about 50% in two days. Parthenin at lethal dose markedly reduced chlorophyll a, b and total chlorophyll contents in water hyacinth leaves. It was interesting that parthenin phytotoxicity dissipated rapidly.

Parthenin appeared to be a potent phytotoxin in an aquatic environment and it was deduced that it might play a decisive role in determining population dynamics and shifts in weed flora in natural ecosystem if it reached the 100 ppm. Sesquiterpene lactones have been reported to be phytotoxic. They react with -SH groups of cysteine, glutathione, and many proteins (Picman 1986). Some have been reported to be novel uncouplers of oxidative phosphorylation (Chefurka 1978).

The mode of action of parthenin on water hyacinth was unknown, but a variety of toxic actions, including some of those previously reported may have combined to cause the stalled growth and development of the weed. Parthenin reduced water use by water hyacinth and caused desiccation of the above-water plant parts consequent to root dysfunction and death. Massive damage to cellular membrane was shown by excessive leakage of solutes from the roots of the treated plants, loss of dehydrogenase activity in roots, and loss of chlorophyll contents in the leaves. These effects suggested that parthenin phytotoxicity may be mediated though affecting macromolecules like proteins, lipids and nucleic acids. The physiological effects of parthenin on water hyacinth resembled those found with *Parthenium* plant residue on water hyacinth and salvinia (Pandey *et al.* 1993a, Pandey 1994a).

Parthenin appears to be a potential herbicidal molecular lead for development of a novel herbicide as it dissipated relatively rapidly in about a month under outdoor conditions (Pandey *et al.* 1993b, Pandey 1994b). The high levels of parthenin in the leaves and flowers largely determine the inhibitory activity of the residue.

Allelochemicals from *Parthenium* for water hyacinth control

A molecule active at 50 ppb to 5 ppm is considered to be of direct commercial herbicidal use. The work undertaken so far showed that *Parthenium* allelochemicals can serve as herbicides for control of water hyacinth at 100 ppm. Since much of stock of secondary metabolites and allelochemicals occurring

in the species have not been investigated so far, it is likely that more potential herbicidal allelochemical molecules or secondary compounds are likely in *Parthenium* and the constituents which are likely to be herbicidal for the aquatic weed water hyacinth at much lower levels than the currently known ones (Pandey *et al.* 1993a, b, Pandey and Mishra 2005, Pandey 1997). It is interesting to note that in these studies the allelochemicals in *Parthenium* appeared to have killed water hyacinth primarily by causing root dysfunction which rendered the treated plants unworthy of keeping pace with the massive evapotranspiratory loss of water and succumbed to desiccation involving massive damage to physiological processes with time leading to death by starving, loss of metabolites and metabolism, in a span of few days (Pandey *et al.* 1993a, Pandey 1996b). This information could be utilized for making the formulations and making them more effective.

Research needs / future thrusts

From the foregoing treatise, it appears that the allelochemicals from *Parthenium* for control of water hyacinth has not been adequately investigated. Our understanding at present has been restricted to display of herbicidal activity of *Parthenium* plant and its parts residue, allelochemical crude and crude fractions obtained from the plant parts by using solvents belonging to a range polarity spectrum (Pandey, unpublished work) and a few phenolics and organic acids and major sesquiterpene lactone parthenin. However, it is obvious from Table 1 and 2 that most of the secondary metabolites and allelochemicals reported from *Parthenium* have not been systematically investigated for herbicidal use, more so for the control of water hyacinth. Investigations on herbicidal allelochemicals in *Parthenium* are essentially needed in the wake of the fact that safer herbicides are rare for the aquatic ecosystems and that herbicides with new modes of action are badly needed due to the rapidly evolving resistance to commercial herbicides, but a new modes of action has not been introduced in over 20 years (Dayan and Duke 2014).

The greatest pest management challenge including for organic agriculture and keeping in view environmental considerations especially in aquatic environment is the lack of effective natural product based herbicides. The structural diversity and evolved biological activity of natural phytotoxins in the plants like *Parthenium* offer opportunities for the development of both directly used natural compounds and synthetic herbicides with new target sites based on the structures of natural phytotoxins. Natural

phytotoxins are also a source for the discovery of new herbicide target sites that can serve as the focus of traditional herbicide discovery efforts. There are many examples of strong natural phytotoxins with new modes of action other than those used by commercial herbicide with new modes of action (Dayan and Duke 2014). Though the allelochemical crude from *Parthenium* leaves show promise for development of a circumstantial herbicidal preparation, yet it needs to be investigated for more effective utilisation of the allelochemicals optimally, which may reduce the rate of application and off course the details of toxicity or toxin persistence would have to be investigated beforehand.

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