SUSTAINABLE WEED MANAGEMENT USING ALLELOPATHIC APPROACH

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ABSTRACT

Allelopathy is a natural phenomenon whereby, the donor plants release chemical compounds (known as allelochemicals) into the environment through decomposition, leaching (caused by rain water), volatilization and root exudates. Allelochemicals from the donor plants can stimulate and/or inhibit the germination and growth of the receiver plants. Allelopathic effects can be categorized based on the following: the effect of the weed on the crop, the effect of the weed on other weeds, the effect of the crop on the weed and the effect of trees on the weed or crop. Thus, allelopathic research can involve several methods such as bioassay, application of plant debris, application of infested soil, the sandwich method, the dish pack method and the plant box method. The allelopathic approach can be applied for controlling weeds through the use of allelochemicals (as natural herbicides) and the allelopathic plants as cover crops/mulch. However, the allelopathic effects of plants depend on biotic and abiotic factors and therefore, more research needs to be carried out to overcome these factors. The allelopathic approach would cause reduction in the dependency on chemical pesticides which are proven contaminants of the environment.

Key words: Allelopathy, donor plants, receiver plants

INTRODUCTION

Pesticide usage is seen as an effective and economic way to control pests, replacing manual animal and mechanical methods of pest control (Jayakumar & Jaganathan, 2007). However, the use of pesticides in agriculture is currently posing problem such as human and crop security, environmental pollution and the evolution of weeds that are resistant to herbicides (Bhadoria, 2011). There are also health risks involved and drinking water sources could become contaminated resulting in negative effects on plants, fish, birds and microorganisms (Mada et al., 2013).

Studies regarding the potential of using the allelopathic phenomenon in agriculture have been gaining momentum among scientists throughout the world, since the seventies (Rice, 1984; Singh et al., 2001). The current orientation of research is more focussed on weed management practices that are safe (for humans) besides being environment-friendly (Bhadoria, 2011). The planning of agricultural activities such as replanting, growing of cover crops and rotational cropping should take into consideration the allelopathic phenomenon which could positively influence agricultural yields (Chon et al., 2006). Thus, plants with allelopathic potential are seen as alternatives that can be used directly or indirectly to control weed growth and overcome the problems that arise from the excessive use of pesticides (Appiah et al., 2015a).

Several bioassay experiments related to allelopathy have been conducted for the last thirty years. These experiments involved the effect of allelopathy on the germination of the test plant species, using plant extracts, plant root exudates and/or plant leachate (Fujii et al., 2007). Thus, several experiments were developed to standardize the experimental methodology for allelopathic research (Fujii et al., 1990). So, the specific method to analyze the allelopathic potential based on the way the allelochemicals are released into environment makes use of agar (containing 0 carbohydrate) as the growing medium of the test
species (Fujii et al., 2007; Mardani et al., 2016). The sandwich method is used to test the allelopathic activity of leaf litter leachate (Morikawa et al., 2012a). The plant box method is used to test the allelopathic activity from the root exudate (Fujii et al., 2007); the dish pack method is used for obtaining the allelochemicals released through volatilization using filter paper as the growing medium of the test species (Fujii et al., 2005).

These specific methods had been developed in order to differentiate and distinguish allelopathic activities apart from other factors such as nutrients, light and water (Fujii et al., 1991). The uncertainty scientist facing is to differentiate between allelopathic activities and competition from other plant species (Fujii et al., 2007).

**PHENOMENON OF ALLELOPATHY**

The term allelopathy was introduced by Hans Molisch in 1937, and it referred to the chemical interaction that takes place among plants, including microorganisms (Weston, 2005). Allelopathy can be defined as the inhibitory and/or stimulatory effect of one plant (including microorganisms) on another plant through the release of chemical compounds into the environment (Rice, 1984). The chemical compounds known as allelochemicals are released into the environment through decomposition, leaching (caused by rain water), volatilization and root exudation (Albuquerque et al., 2011; Rice, 1984). Albuquerque et al. (2011) reported that allelochemicals from the donor plant can affect the growth and development of the receiver plant (seedlings) through changes in physiological and biochemical processes.

Allelochemicals can be obtained from several parts of the donor plant, namely, the leaves, flowers, fruits, stems, roots and buds. Allelochemicals are less toxic compared to chemical pesticides (Khanh et al., 2008). Allelochemicals are produced from secondary metabolism and are non-nutritional primary metabolites (Iqbal & Fry, 2012). Li et al. (2010) classified allelochemicals into 10 categories, namely (1) water soluble organic acids, straight-chain alcohols, aliphatic aldehydes and ketones, (2) simple lactones, (3) long-chain fatty acids and polyacetylenes, (4) quinines, (5) phenolics, (6) cinnamic acid and its derivatives, (7) coumarins, (8) flavonoids, (9) tannins and (10) steroids and terpenoids. The types of allelochemicals isolated from plants are summarized and listed in Table 1.

Khanh et al. (2008) listed approximately 18 compounds present in soil infested with *Echinochloa crus-galli* as allelochemicals (Table 1). Studies conducted found that sorgoleone (Table 1) at 100 μM inhibited the growth of *Solanum nigrum* L. and *Ambrosia artemisiifolia* L. by 80% (Nimbal et al., 1996). Momilactones at concentrations higher than 10μM inhibited the growth of *Echinochloa colona* (L.) Link, a common weed found in rice fields (Chung et al., 2005).

Potential allelopathic plants are usually selected based on specific criteria such as being of medicinal value and being invasive. The plants of medicinal value are easier to screen possibly

**Table 1. Allelochemicals in weeds**

<table>
<thead>
<tr>
<th>Plants</th>
<th>Allelochemicals</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Centaurea maculosa</em> Lam.</td>
<td>Catechin</td>
<td>Bais et al., 2002</td>
</tr>
<tr>
<td><em>Echinochloa crus-galli</em> (L.) Beauv</td>
<td>Terpene, cinnamic acid, ferulic acid, fatty acids and steroids</td>
<td>Khanh et al., 2008</td>
</tr>
<tr>
<td><em>Sambucus nigra</em> L.</td>
<td>Cyaanogenins, lignans, flavonoids and phenolic glycosides</td>
<td>D’ Abrosca et al., 2001</td>
</tr>
<tr>
<td><em>Leonurus sibiricus</em> L.</td>
<td>Phenolics and caffeic acids</td>
<td>Mandal, 2001</td>
</tr>
<tr>
<td><em>Conyza canadensis</em> L.</td>
<td>Gallic acid, vanillic acid, catechol and syringic acid</td>
<td>Shaukat et al., 2003</td>
</tr>
<tr>
<td><em>Artemisia annua</em> L.</td>
<td>Artemisinin</td>
<td>Nguyen et al., 2011</td>
</tr>
<tr>
<td><em>Sorghum bicolor</em> L. Moench</td>
<td>Sorgoleone</td>
<td>Czarnota et al., 2003</td>
</tr>
<tr>
<td><em>Oryza sativa</em> L.</td>
<td>Morimlaactone</td>
<td>Kato-noguchi et al., 2010</td>
</tr>
<tr>
<td><em>Callistemon citrinus</em> Curtis</td>
<td>Leptostermone</td>
<td>Corne, 2006</td>
</tr>
<tr>
<td><em>Piper longum</em> L.</td>
<td>Sarmentine</td>
<td>Huang et al., 2010</td>
</tr>
</tbody>
</table>

Fig. 1. The pathway of allelochemicals released into the environment (modified from Albuquerque et al., 2011; Chon et al., 2006).
because they have more bioactive compounds compared to those in the other plants (Islam & Kato-Noguchi, 2013). Studies by Fujii et al. (1991) showed that plants of medicinal value have strong allelopathic activity. Invasive plants on the other hand are selected because of their ability to dominate the vegetation within specific areas because of the presence of allelopathic compounds and also because of their function as mediators in interactions between plants (Callaway & Ridenour, 2004). To date, studies on plants that are able to prevent other plants from growing around them are gaining momentum and there is the possibility that chemical compounds from these plants can be used as selective bioherbicides (Duke, 2010).

ALLELOPATHIC EFFECTS

Allelopathic effects among plants can be observed as follows: the effect of the weed on the crop, the effect of the weed on other weeds, the effect of the crop on the weed and the effect of trees on the weeds and/or crops. Studies conducted by Nornasuha & Ismail (2015) showed that the aqueous leaf extract of Chromolaena odorata and Mikania micrantha (two weed species) at the concentration of 50 g/L inhibited more than 50% (compared to that of the control) of the seedling growth of Brassica chinensis var. parachinensis (a vegetable crop). The aqueous leaf extract at the concentration of 50 g/L and the leaf debris at the rate of 10 g leaf debris/ 500 g soil of C. odorata and M. micrantha also significantly inhibited the growth parameters of Eleusine indica and Ageratum conyzoides (two weed species) (Ismail & Nornasuha, 2014).

The allelopathic effect of the crop on the weed can be seen from studies by Ismail et al. (2016) where Pueraria javanica (leguminous cover crop) at the rate of 10 mg and 50 mg leaf litter leachate significantly inhibited the radicle length of E. indica, C. iria and C. odorata (three weed species). The leaf extract of Pueraria javanica at the concentration of 66.7 g/L inhibited by more than 90% (compared to that of the control) of the shoot and radicle length, the fresh weight and the germination percentage of E. indica and C. odorata (Ismail et al., 2016). Allelopathic effects can also be observed from the effect of trees on weed growth. Studies by Ishak et al. (2016) showed that at the concentration of 66.7 g/L, the seed extract of Leucaena leucocephala (tree species) inhibited more than 50% (compared to that of the control), the germination of Ageratum conyzoides, Tridax procumbens and Emilia sonchifolia.

RESEARCH ON ALLELOPATHY

Bioassay method

The method of observing the germination and growth of the bioassay species in petri dishes is the common method for studies on allelopathy. This method is used to explore the effect of the allelopathic plant on the germination and growth of other plant species (known as the bioassay species) (Albuquerque et al., 2011). The germination percentage differed depending on the different types of extract used. The extracts were produced from different types of samples such as fresh samples, dry samples, powdered samples as well as plant debris and soil infested with allelopathic plants and diluted to different concentrations. In the germination test too, different type of growth media such as filter paper, agar, sand and soil (Allan, 2012) were used. The commonly used bioassay species is Lactuca sativa. As it germinates very fast and uniformly, and is very sensitive to different types and concentration of extracts (Akhtar et al., 2014). Macias et al. (2000) suggested the use of crop species from families that are different from that of the bioassay plant species. Xuan et al. (2004a) however, recommended the use of weeds as the bioassay species in order to investigate the selective properties of the allelochemicals. The weed species can be selected based on morphology of the weed and the level of its invasiveness in the plantation areas.

Application of plant debris

The debris of allelopathic plants can be tested through incorporation of the plant debris in different quantities into the substrate in order to determine the effect of allelopathy on the decomposition process and the amount/type of allelochemicals released on the target plant species. The experiment when conducted in the green house as well as in the field, is able to demonstrate the allelopathic processes that occurs (Albuquerque et al., 2011). When the debris of Parthenium hysterophorus was incorporated at the rate of 40 g / 1000 g soil, it resulted in decrease in the size and dry weight of Brassica oleracea, B. campestris and B. rapa. This reaction occurred because the phenolic compounds which are soluble in water, were released by P. hysterophorus (Singh et al., 2005). Meanwhile, soil that had been incorporated with Chenopodium murale has been reported to inhibit the germination, nodulation and macromolecule content of legume plants namely Cicer arrietinum and Pisum sativum (Batish et al., 2007).
Allelochemical residue in soil

A study was undertaken to investigate the effect of using soil infested with allelopathic plant material as the growth medium of the target plant species (Kruse et al., 2000). Soil which was not infested with allelopathic plant material was used as the control. The aqueous extract from the infested soil was also tested using the bioassay method (Albuquerque et al., 2011). Studies by Tongma et al. (2001) showed that soil infested with Tithonia diversifolia for five years, inhibited the germination and growth of six target plant species. Meanwhile, Khanh et al. (2008) reported that paddy soils infested with Echinochloa crus-galli inhibited the growth of the rice plants (Oryza sativa).

Sandwich method

The sandwich method is used to investigate the potential allelopathic effect of the leaf litter leachate of allelopathic plants in the laboratory (Fujii et al., 2003). This approach is one of the bioassay methods which saves time and can be used to screen plant samples on a large scale (Morikawa et al., 2012b). The oven dried leaf will be placed at the bottom of a six-well multi-dish. Then, autoclaved agar will be prepared and cooled to a temperature of 40ºC – 45ºC, prior to being poured into the six-well multi-dish. When the agar solidifies, another layer of agar will be poured into the same six-well multi-dish. This will create two layers of agar with the plant sample between them. The seeds of the target plant species will then be sown on the surface of the second layer of agar. This will provide the physical barrier between the tested species and the leaf sample (Mardani et al., 2016). The six-well multi-dish will be sealed and incubated under controlled conditions (Appiah et al., 2015a). Appiah et al. (2015b) used this sandwich method to screen 251 plant species in the Sino-Japanese floristic region in order to study their allelopathic activities.

Dish pack method

The dish pack method is carried out to study the allelopathic activity from leaf volatilization under controlled conditions. This method uses the six-well multi-dish with 2 g of the fresh leaf sample or 200 mg of the oven dried leaf samples placed into one well. The other five wells will be sown with the seeds of the bioassay species using filter paper as the growth medium. The filter paper will be moistened with 0.7 mL distilled water. Then, the multi-dish will be sealed and incubated under controlled conditions (Appiah et al., 2015b). The allelopathic effect from leaf volatilization on the bioassay species will be measured based on the

\[\text{Fig. 2A. A diagram of the sandwich method. In the sandwich method the position of the allelopathic plant species (donor plants) is situated between two layers of agar (A).}\]

\[\text{Fig 2(B). Shows the position of the control in the multi-dish.}\]

\[\text{Fig. 3. A diagram of the dish pack method. In this method, (a), (b), (c) and (d) represent the distance of the bioassay species seeds from allelopathic plant species (donor plant) @ 41 mm, 58 mm, 82 mm and 92 mm, respectively.}\]
distance of the bioassay species (receiver plant) from the well filled with the leaf sample (donor plant) (Fujii et al., 2005).

**Plant Box method**

The plant box method was developed based on the dose response principle where the distance of the donor plant to the bioassay species (receiver plant) is used. It is related to the inhibitory process that occurs in the receiver plant due to the concentration of the root exudate into the growth medium. In this method agar is used as the growth medium and this allows the allelochemicals to move from the root of the donor plant to that of the bioassay species. The bioassay species that showed higher inhibitory percentages were the ones that were sown nearer to the root of the donor plant (Fujii et al., 2007). This method was used by Appiah et al. (2015a) to study the allelopathic activity of selected *Mucuna pruriens* genotype. Besides, it has also been used to identify the allelopathic activity of 19 medicinal plants of Pakistan (Syed et al., 2014). The root exudate of *Sarcococca saligna* exhibited the highest inhibition compared to other 18 species by causing reduction in the radicle growth of *Lactuca sativa* by 78% (Syed et al., 2014).

**ALLELOPATHIC APPROACH**

**The use of allelochemicals as bio herbicides**

The allelochemicals have a mode of action similar to that of herbicides (Soltys et al., 2013). Most of the allelochemicals are partially or completely water soluble. Thus, it is easier to apply them without using surfactants (Dayan et al., 2009). This is different from the use of fungi as bio herbicides because the life span of the bio herbicides from fungi is shorter and specific environment application procedures are required e.g. some fungi need water and dew to react (Hoagland, 2001). Besides, allelochemicals are reported to have less halogen atoms, complex chemical structures and short half-life in the ecosystem (Duke et al., 2002). Allelochemicals are naturally produced compounds which are environmentally friendly and safer compared to chemically produced pesticides.

Some synthetic herbicides have been developed, analogous to the plant chemical compounds. For example, the most popular synthetic herbicide; 2,4-dichlorofenoxy acetic acid (2,4-D); is analogous to the hormone auxin (auxin is a plant growth hormone). The herbicide 2,4-D is used to control broad leaved weeds and has been widely used and commercialized (Zimdahl, 2007). In addition, the triketone herbicide, Callisto™, was developed to control broad leaved weeds in maize fields (Rimando & Duke, 2006). The active ingredient of Callisto™ is mesotrione, which is analogous to leptospernone (allelochemical that can be found in *Callistemon* species). In the field trials, 9000 g ai ha⁻¹ leptospernone was needed to control weed species. As this amount of the chemical compound is difficult to obtain, it led to the development and commercialization of the herbicide (CORNES, 2005).

There have been cases where allelochemicals were applied directly as natural herbicides. For example, Organic Interceptor® (@680 g/litre pine essence) is a non- selective contact herbicide that is obtained from the residue of the liquid recovered during the processing of pulp and paper from pine trees. This herbicide can disrupt membrane permeability and contribute to rapid dehydration within a few days (Allan, 2012; James et al., 2002). The BioWeed® herbicide is also a plant-derived product and is a non systemic, pre emergence herbicide. The active compound of this herbicide is alpha terpineol (active compound from pine trees). This herbicide acts by stripping the outer wax layer of the leaf surface and then causing the weed to...
become dehydrated. It is used to control the germination of weed seeds on the soil surface, as well as in combination with glyphosate for sustainable weed control (Allan, 2012).

Extracts from allelopathic plants can be used as foliar sprays. Applications of the aqueous extracts of sorghum (Sorghum bicolor also known as sorgaab) and sunflower (Helianthus annuus L. also known as sunfaag) (Soltys et al., 2013) have been reported. Cheema and Khaliq (2000) sprayed sorgaab the aqueous extract of Sorghum bicolor to control weeds in Triticum aestivum fields. They found that sorgaab could inhibit by 40 to 50% the germination of weed seeds. Studies were also carried out to investigate whether the aqueous extract of S. bicolor needed to be combined with other allelopathic plant extracts in order to control weeds effectively (Mahmood et al., 2009; Jabran et al., 2010). These studies indicate that weeds can be effectively controlled with sorgaab in combination with other aqueous plant extracts and low concentrations of the pendimethalin herbicide (Jabran et al., 2010).

**Allelopathic plants as cover crops**

Cover crops are plant species which are not the main crop but are introduced in cropping, especially at times when the soil is left idle and not cultivated. In addition, cover crops are used in non cultivated areas, in order to prevent erosion and to conserve the moisture and nutrients in the soil (Gallandt et al., 1999). Cover crops are also important in the rotational cropping system because these plants grow rapidly and can form a cover on the soil surface that can prevent the germination and growth of weed species (Singh et al., 2001). The use of allelopathic cover crops that can inhibit the growth of other plants has been suggested by Fujii (2003) as one of the effective ways in the integration of the allelopathic concept in weed control. Fujii (2001) tested 53 species of plant cover crops using the plant box method. The study indicated that certain cover crops have the potential to be used to control weeds. These lower crops included Avena sativa, Hordeum vulgare, Secale cereal, Mucuna pruriens and Vicia villosa. Vicia villosa was able to control weeds in the paddy fields by forming a thick cover on the paddy soil surface when the fields were dry during the summer season (Fujii, 2001).

**Allelopathic plants as mulching materials**

Plant residues, plant cover crops and plant mulch are always being used in weed control management activities as they can be obtained in large quantities from the field. Mulching can act as a physical barrier in reducing the amount of sunlight, temperature and moisture which are very important for weed seed germination (Davies et al., 2008). The effectiveness of weed control through mulching increases when the mulch control through mulching increases when the mulch control through mulching increases when the soil population and 75% of the dry weight of weeds compared to that of the control. The weeds that were controlled included Graticola japonica, Lindernia pyxidaria, Echinochloa oryzicola, Eleocharis acicularis, Monochoria vaginalis and Rotala indica (Xuan et al., 2004b).

**CHALLENGES IN USING THE ALLELOPATHIC APPROACH**

**Abiotic factors**

The concentration of allelochemicals in the donor plant can be influenced by several environmental factors which include temperature, light, soil structure and soil nutrient content. The quantity of allelochemicals has been reported to increase when the donor plant is grown in a habitat that has low to moderate soil nutrients content (Bhowmik & Inderjit, 2003). Increment in the production of phenolic compounds was also reported when there are changes in soil chemical properties such as pH and conductivity (Batish et al., 2002), change in light quality (Johnson et al., 1997) and with the application of herbicides on the donor plant (Santosh et al., 1999). Tongma et al. (2001) reported that allelopathic activities of plants that grow under dry soil conditions are higher than those of plants that grow in well irrigated areas. All of these reactions depend on the plants’ reaction to stress conditions. Plants release more allelochemicals when stressed (Einhellig, 1999).

The physical and chemical properties of the soil influence the movement of allelochemicals from the root of the allelopathic plant to the root of the receiver plant. This was observed when the leaf leachate of Pluchea under clayey, sandy, and loamy soil conditions, contained different amounts of phenolic compounds (Inderjit & Dakshini, 1994). Some of the physical and chemical properties of soils involved in allelopathic interactions include soil texture, pH, organic carbon content and soil nutrient content (Jayakumar & Jaganathan, 2007).

Allelopathic activity in the environment is influenced by the plants. The existence of high population density of the receiver plant species can influence allelopathic activity because only low concentrations of allelochemicals reach each receiver plant. Besides, allelopathic activity can also be influenced by differences in the life cycle
patterns of the donor and receiver plants (e.g. time of planting, time of germination). Crop yield can be increased when the weeds germinate after the crop is established. The growth stage, habit and habitat of the donor plant influences the amount of allelochemicals released into the environment (Jayakumar & Jaganathan, 2007).

Biotic factors
Allelopathic activity can also be influenced by pathogenic organisms. When donor plants are exposed to pathogenic organisms such as herbivores, the plants react by increasing the defense mechanism through increment in the production of allelochemicals and synthesis of secondary metabolites. Indirectly, the allelopathic activity will increase (Einhellig, 1999). This was observed when Lolium perenne was infected by the fungus, Acremonium lolii, which can inhibit the growth of Trifolium repense (Sutherland et al., 1999).

Biotic stress will be produced when plant debris reacts with soil microorganisms during the decomposition process and affects the growth of new plants. During the decomposition process, soil microorganisms use the available energy sources, water and oxygen leading to deficiency of these essential resources. Even though the decomposition process provides nutrients to the soil, allelochemicals which are phytotoxic will simultaneously be produced. The situation will become serious when the plant debris is exposed to environmental stress like high temperature, leading to high concentration of allelochemicals produced (Einhellig, 1999). When the soil contains high amounts of allelochemicals, chemical stress will occur as high amounts of allelochemicals will be absorbed by the receiver plant, due to the uptake and the “de novo” synthesis process of the receiver plant. Inderjit and Dakshimi (1992) reported that the phenolic content in Vigna unguiculata var. sesquipedalis was higher compared to that of the control when sown in soil which was incorporated with the debris of Pluchea lanceolata. Autotoxicity is a type of biotic stress that occurs within plants of the same species. The existence of autotoxicity contributes to the replanting problem of field crops and crops which need to be replanted every year (Singh et al., 1999). Competition is also a type of biotic stress. When the allelopathic plant competes with other plants for available resources or the allelopathic plant grows under unsuitable conditions, biosynthesis of allelochemicals will be stimulated and this will have negative effects on the growth of the surrounding plants (Einhellig, 1999).

CONCLUSION
Application of the allelopathic approach as one of the strategies in weed control provides an alternative methodology for underdeveloped and developing countries to establish a sustainable and environmental-friendly agricultural system. However, in order to ensure that the allelopathic approach is successful it should be simple and economically viable. This can be achieved with the use of local plants with allelopathic properties and which are widespread and easily available. Recent advances include the ability to isolate and identify bioactive substances, known as allelochemicals. Further research is needed to develop and make them useful compounds in weed control. However, a multidisciplinary approach is needed to assess the allelopathic influence and plant interactions.

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