

## 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

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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

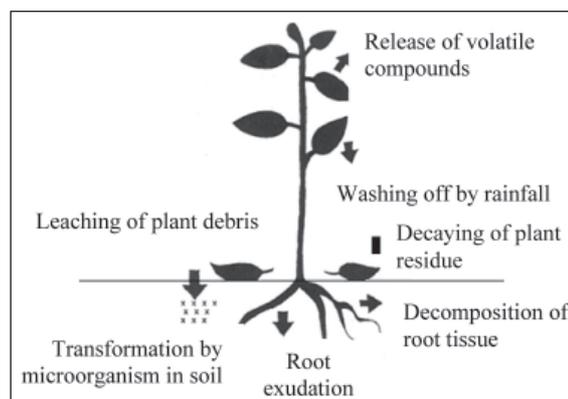
The term allelopathy was introduced by Hans Molisch in 1937, and it referred to the chemical interaction that takes place among plants, including microorganisms (Weston, 2005). Allelopathy can be defined as the inhibitory and/or stimulatory effect of one plant (including microorganisms) on another plant 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 (Khan *et al.*, 2011). 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  $\mu\text{M}$  inhibited the growth of *Solanum nigrum* L. and *Ambrosia atrtemisiflora* L. by 80% (Nimbal *et al.*, 1996). Momilactones at concentrations higher than 10 $\mu\text{M}$  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



**Fig. 1.** The pathway of allelochemicals released into the environment (modified from Albuquerque *et al.*, 2011; Chon *et al.*, 2006).

**Table 1.** Allelochemicals in weeds

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

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 arietinum* 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*).

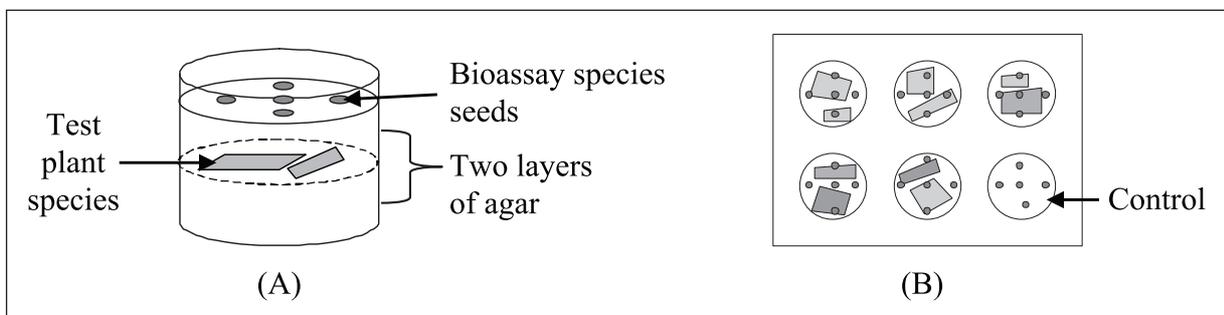
### Sandwich method

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 40°C – 45°C, 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.

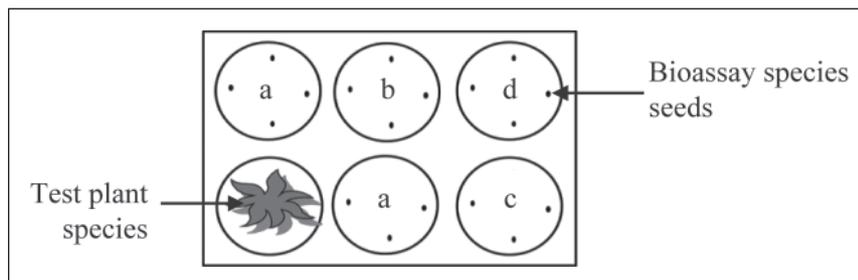
### Dish pack method

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

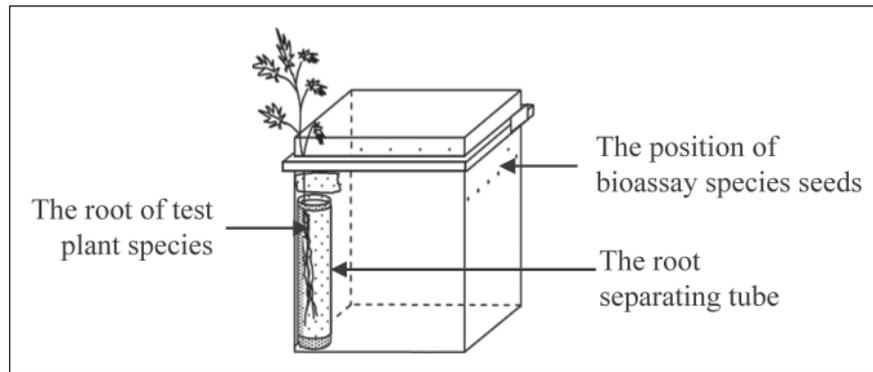


**Fig. 2A.** A diagram of the sandwich method. In the sandwich method the position of the allelopathic plant species (donor plants) is situated between two layers of agar (A).

**Fig 2(B).** Shows the position of the control in the multi-dish.



**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.



**Fig. 4.** The diagram of plant box method. The root of the allelopathic plant species (donor plant) was placed in the root separating tube. The bioassay species seeds were sown at different distances from the root of the donor plant (Fujii *et al.*, 2007).

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 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-dichlorophenoxy 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 leptospermone (allelochemical that can be found in *Callistemon* species). In the field trials, 9000 g ai ha<sup>-1</sup> leptospermone 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 plants used possess allelopathic properties. This was observed when *Ageratum conyzoides* was used as mulch at the rate of 2 t ha<sup>-1</sup> in the paddy field. This weed inhibited approximately 86% of the weed 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 lanceolate*. 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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