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Phytotoxic effects of *Pueraria javanica* litter on growth of weeds *Asystasia gangetica* and *Pennisetum polystachion*

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ABSTRACT

Allelopathic effects of *Pueraria javanica* cover crop grown under oil palm and its litter on control of weeds (*Asystasia gangetica* and *Pennisetum polystachion*) in oil palms was explored in laboratory bioassay and potculture. In Lab bioassay, the litter leachates of *P. javanica* significantly reduced the seed germination (%) and delayed the seed germination of *Asystasia gangetica* but did not affect the germination (%) and seed germination time of *Pennisetum polystachion*. *Pueraria javanica* leachates significantly reduced the radicle lengths of both *A. gangetica* and *P. polystachion* seedlings. The inhibitory effect was higher on *P. polystachion* growth, *P. javanica* at the highest concentration (50 g L^{-1}) caused 43% reduction in radicle length than 23% reduction in *Asystasia gangetica*. However in pot culture, the increasing concentrations of *P. javanica* leachate-amended soils did not affect the root and shoot lengths, dry weight and chlorophyll concentration of *A. gangetica* and *P. polystachion* seedlings. The decomposition study of *P. javanica* in soil showed that the phenolic compounds in *P. javanica* litter did not remain stable in soil for > 6 weeks. The allelopathic effects of *P. javanica* litter on germination depended on weed species, but *P. javanica* litter did not interfere with seedling growth of test weed species. The allelopathic activity did not persist for long periods of time in soil.

Key words: Allelopathy, *Asystasia gangetica,* bioassay, germination, litter leachates, *Pennisetum polystachion*, phenolic compounds, *Pueraria javanica,* seedling growth.

INTRODUCTION

Many plant species, including crop plants, are capable of producing and releasing biologically active compounds (allelochemicals). Allelochemicals (e.g. phenolics, terpenoids, alkaloids, coumarins, tannins, steroids and quinines) are released by plants into the environment by leaching from the leaves and other aerial parts, volatile emissions, root exudation and the decomposition of plant materials (35,40). Allelopathy is considered an attractive method for weed management due to its environmental friendliness (41). Initially allelopathic studies were mainly done using leachates or extract in bioassays without soil (21). However, from 1990's, allelopathic research shifted from merely laboratory work to field studies (7). The interaction of allelochemicals with soil

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components upon release from the plant is important in determining whether inhibition of the target plant is likely to occur in the field (2,3). Phenolic compounds are ubiquitous in plant materials, and in many allelopathic studies, phenolic compounds in plant extracts were correlated most strongly with growth inhibition of several plants (15,25). Phenolic compounds are rapidly adsorbed and/or oxidized by soil (9,19,22). To fully exploit the phytotoxicity of cover crops for weed management, an understanding of the role of soil in allelopathic process in fields is required (8).

In Malaysia since sixties conventional legume cover crops (LCC) such as *Centrosema pubescens, Pueraria phaseoloides* (*P. javanica*) and *Calopogonium caeruleum* are cultivated under D × P oil palms to preserve the fertility and productivity of fragile soil (5). Tropical *P. javanica* is native to the Malaysian and Indonesian region, with green fodder yields up to 30-50 t/ha (dry matter yields 4-10 t/ha) (11). Cover crop species besides competing with weeds for growth resources (water, nutrients and light) may also have allelopathic effects. Several studies on the allelopathic potential of legumes and non-legumes have been published (6,12,17,23), but the allelopathic effects of conventional LCC were not examined. Some sporadic attempts were made to assess the phytotoxicity of LCC (1,4,33). However, most bioassay studies only investigated the toxicity of leaves and the chemicals were isolated from the leachates or leaves.

Hence, it was hypothesized that phenolic compounds may be released into the environment from *P. javanica* litter and subsequently cause allelopathic interference among its competitors. To test this hypothesis, this study was designed: (i) to determine under laboratory conditions, the effects of phytotoxic litter of *P. javanica* on the germination of two major weeds (*Pennisetum polystachion* and *Asystasia gangetica*) in oil palm plantations (37), (ii) to evaluate if the *P. javanica* litter retains allelopathic potential in soil for how much time and (iii) to determine the changes in the levels of water-soluble phenolics in the soil.

MATERIALS AND METHODS

Collection of plant materials

Mature seeds of weeds were collected from 50 plants of *A. gangetica* and 20 plants of *P. polystachion* from the same D × P oil palm fields in May 2011. In September 2011, the litter of *Pueraria javanica* plants was collected from the top layer of litter beds of 1-year old plants from D × P oil palm fields (3° 02' N, 101° 42' E; elevation 31 m a.s.l.). In laboratory, the un-decomposed and partially decomposed litter consisting of distinguishable fallen leaves, petioles and branchlets, were separated from the litter of other species, mineral soil and humus. As the chemical nature of allelochemicals alters at high temperatures, the litter was shade-dried at room temperature (25 ^{o}C) for 10 days. The litter was then ground to powder and stored for 1 month in polyethylene bags until used.

Preparation of leachates

The ground *P. javanica* litter (100 g) was steeped in 1 L distilled water for 18 h at room temperature (25 °C), and filtered through a double layer of cheesecloth, followed by filtration through filter paper (No.1, Whatman). The extract was diluted with distilled water to get 10, 20, 30, 40 and 50 g L^{-1} concentrations, these were kept in refrigerator at 4 °C until used. Distilled water was used as control (0 g L^{-1}).

Experiment I. Litter leachates- Bioassay

Healthy weed seeds of uniform size were pre-treated with 1.5% (v/v) sodium hypochlorite solution for 1 min for surface sterilization and then washed thrice for 3 min with distilled water. This treatment did not inhibit seed germination. Five ml of each litter leachate (10, 30, or 50 g L^{-1}) or distilled water (for control) were added to 9 cm dia sterile (Petri dishes lined with two sterile filter papers (No.2, Whatman). These dishes were kept at room temperature for 2 h to ensure that the temperature of solution was in equilibrium with room temperature. Then, 25-surface-sterilized seeds of *A. gangetica* or *P. polystachion* were placed in each petri dish. The petri dishes were sealed with parafilm to prevent water loss and avoid contamination and incubated at room temperature (25 °C) for 9 days in dark. The experiment was done in a completely randomized design (CRD) with four replications. Germination was considered when a radicle of least 1 mm protruded beyond the seed coat. The number of weed seeds germinated daily in all Petri dishes were counted till 9 days. The length of radicles and hypocotyls were measured after 9 days.

Final germination percent (FGP) and mean germination time (MGT) were calculated as under (32):

$$\text{FGP} \quad (\%) \quad = \frac{\text{Nt} \ \times 100}{\text{N}}$$

Where, Nt: Final number of germinated seeds in respective treatments. N: Number of seeds used in bioassay.

$$\text{MGT} \quad (\text{days}) \ = n1 \times D1 + n2 \times D2 + n3 \times D3 + ... \ / \ \text{Final number of seeds germinated}$$

Where, n: Number of germinated seeds and D: Number of days.

The % reduction in germination, mean germination time, root length and shoot length were determined as under:

$$\text{Percent} \quad \text{reduction} \quad = \frac{C - T}{C} \times 100$$

Where, C: Respective value in control treatment and T: Respective value in treatment.

Experiment II: Litter leachates- Pot culture

The Expermental treatments consisted of two factors: (i). *P. javanica* leachate concentrations: 6 (0,10,20,30,40,50 gl^{-1}) and (ii). Weed spp.: 2 (*A. gangetica* and *P. polystachion*). The experiment was conducted in 15×5 cm polybags in March 2012. The polybags were kept in glasshouse [25 °C minimum and 32 °C maximum, 95 ± 2 % relative humidity, and a 12/12 h light/dark regime].

Soil for this experiment was collected from the oil palm field. Ten *P. javanica* -free spots were selected and one soil sample (0-20 cm) was taken from each spot. A composite soil mass was made by mixing the individual soil masses thoroughly. The soil was then crushed, sieved through 2 mm sieve and air-dried. The soil was sandy clay loam (45.19% clay, 11.35% silt and 43.22% sand, pH=4.69, CEC= 6.4 cmol kg^{-1}, total N= 0.12%, available P= 4.1ppm, exchangeable K= 31ppm, exchangeable Ca= 68.3ppm, exchangeable Mg= 49.3ppm and organic carbon= 1.4%). Two hundrd and fifty g soil was placed in each polybag.

The *A. gangetica* and *P. polystachion* seeds were allowed to sprout and germinate for 3-days at room temperature in dark**.** The 3-day-old weed seedlings were then transplanted into the polybags with 10-seedlings per polybag. After transplanting, 50 ml litter leachate was applied per polybag as per treatments. The root and shoot lengths and seedling dry weights were recorded after 2-weeks. The *P. polystachion* shoot length (cm) was measured from the ground level to the tip of longest leaf. The *A. gangetica* shoot length (cm) was measured from the ground level to the tip of shoot. Shoot and root samples were carefully separated and rinsed in water and oven-dried at 70 ^{o}C for 72 h. The total dry matter was calculated as total of root and shoot weights.

Leaf chlorophyll content was estimated using the method of Witham *et al*. (38). Fresh leaf from each polybag was cut into pieces using scissors and 200 mg of cut leaves were transferred into a plastic vial containing 20 mL of 80% acetone. The vial was quickly corked airtight and kept in dark for 72 h. Absorbency of solution was recorded at 645 nm and 663 nm using a scanning spectrophotometer (Model UV-3101PC, UV-VIS NIR). Chlorophyll content was estimated and expressed as mg g^{-1} of sample as under:

$$\text{Chlorophyll a content (mg g}^{-1}\text{ fresh leaf)} = \frac{12.7(A_{663}) - 2.69(A_{645})}{1000} \times \frac{V}{W}$$

$$\text{Chlorophyll b content (mg g}^{-1}\text{ fresh leaf)} = \frac{22.9(A_{645}) - 4.86(A_{663})}{1000} \times \frac{V}{W}$$

$$\text{Total chlorophyll content (mg g}^{-1}\text{ fresh leaf)} = \frac{20.2(A_{645}) + 8.02(A_{663})}{1000} \times \frac{V}{W}$$

Where, A645: Absorbance of the solution at 645 nm, A663: Absorbance of the solution at 663 nm, V: Volume of the solution in mL, W: Weight of fresh leaf sample in gram. The absorption coefficients were 12.7, 2.69, 22.9, 4.86, 20.2 and 8.02

Experiment III. Fate of *P. javanica* litter phenolics in the soil

The experiment was done in 100 cm^2 plastic containers in glasshouse (similar to Experiment 2). Five g *P. javanica* litter was mixed thoroughly with 250 g soil (collected from oil palm field) and placed in 100 cm^2 plastic containers. To facilitate decomposition of *P. javanica* litter, 15 mL microbial inoculant was added to each container. The microbial inoculant was prepared by incubating 150 g fresh soil collected from a cultivated field with 10 mL of Hoagland's solution and 15 mL of distilled water in dark for 4 days. Then, 300 mL distilled water was added and the supernatant was filtered through Whatman No. 1 filter paper (26). The containers were covered with perforated aluminum foil and incubated in dark in glasshouse. The samples were re-adjusted gravimetrically to their initial water content at weekly intervals. One set of 3-pots was removed each time for chemical analysis at 0, 1, 2, 3, 4 and 5 weeks after incubation.

All 250 g soil from each container was extracted by adding 250 ml distilled water and shaking for 1 h at room temperature, and filtering the extract through Whatman No. 1 filter paper. The extracts were preserved in refrigerator at 4 °C. The amount of phenolics in water extracts were estimated using the Folin-Ciocalteu assay. For this assay, an aliquot of 1.0 mL of soil extract was placed into a test tube and 5 mL of 2% Na_2CO_3 in 0.1 N NaOH was added and mixed with a test-tube mixer. Five min later, 0.5 mL of Folin-Ciocalteu reagent was added and the solution was mixed again. The absorbance was read using a spectrophotometer (Model UV-3101PC, UV-VIS NIR) at 760 nm after 2 h. A standard curve, 25 ppm until 500 ppm depending on the concentration of phenolic compounds in the experiment, was prepared in a similar manner using a concentration series of gallic acid solutions in water and the phenolic concentration in the soil extracts was then estimated (as gallic acid equivalent), based on this standard curve. To estimate the water-soluble phenolics in *P. javanica* tissues (litter and shoot) 5 g plant tissue was extracted with 50 mL distilled water (30). To estimate the acetone extractable phenolics in *P. javanica* tissue or soil samples, the same protocol was used (except for the extraction). The extracts were prepared using 70% acetone.

VI. Statistical analysis

Regression analysis was performed to determine the relationship among variables and treatments using the Sigmaplot software version 11.

RESULTS

The litter leachates of *P. javanica* decreased the germination of *A. gangetica* seeds (Fig. 1). The germination decreased quadratic polynomially with increasing litter leachate concentrations. The differences were statistically significant (P= 0.05). However there was no effect on germination of *P. polystachion*. With increasing litter leachate levels, the germination of *A. gangetica* was delayed (Fig. 2). The longest delay (6.64 days) in its germination occurred at the highest leachate concentration (50 g L^{-1}), followed by 30 (6.14 days), 10 (5.9 days) and 0 g L^{-1} (5.7 days) (Fig. 2). The germination of *P. polystachion* was not delayed by *P. javanica* leachates. Increasing the litter leachate levels of *P. javanica* decreased the hypocotyl length of *A. gangetica* and *P. polystachion* seedlings (Fig. 3), but differences were not significant (P = 0.41 and P= 0.45, respectively). *P. javanica* litter leachate concentrations decreased quadratic polynomially the radical length of *A. gangetica* (P= 0.05) (Fig. 4). The radical length of *P. polystachion* decreased linearly with increasing litter leachate concentration (Fig. 4, P= 0.01). The R-values in both species suggested that the correlation between the leachate concentration and radical length was high. The litter leachates of *P. javanica* decreased the radical length of *A. gangetica*, in following order: 50 g L^{-1} (23%) > 30 g L^{-1} (18%) > 10 g L^{-1} (3%). The effects of *P. javanica* leachates on *P. polystachion* radicle length was more conspicuous with 50 g L^{-1} concentration. The 50 and 30 g L^{-1} litter leachate concentrations of

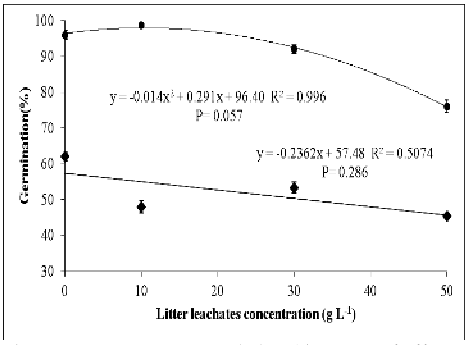

Figure 1. Dose–response relationship curve of effect of *P. javanica* litter leachates on final germination (%) of *A. gangetica* (●) and *P. polystachion* (♦). The error bars are standard deviations (SD) and the bars represent the media of four replicates.

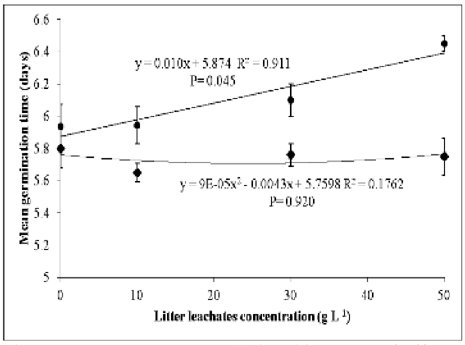

Figure 2. Dose–response relationship curve of effect of *P. javanica* litter leachates on mean germination time of *A. gangetica* (●) and *P. polystachion* (♦). The error bars are standard deviations (SD) and the bars represent the media of four

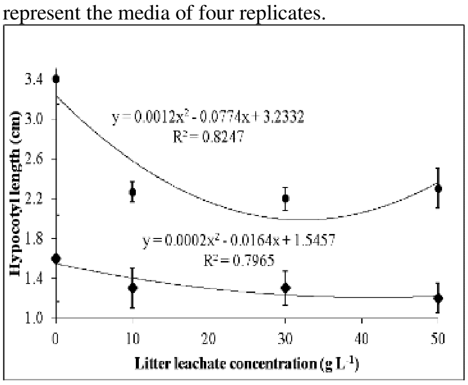

Figure 3. Dose–response relationship curve of effect of *P. javanica* litter leachates on hypocotyl length of *A. gangetica* (●) and *P. polystachion* (♦). The error bars are standard deviations (SD) and the bars represent the media of four replicates.

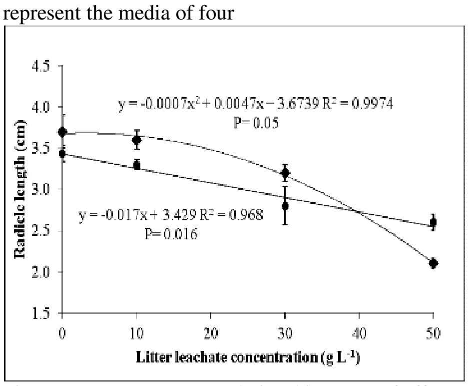

Figure 4. Dose–response relationship curve of effect of *P. javanica* litter leachates on radicle length of *A. gangetica* (●) and *P. polystachion* (♦). The error bars are standard deviations (SD) and the bars represent the media of four replicates.

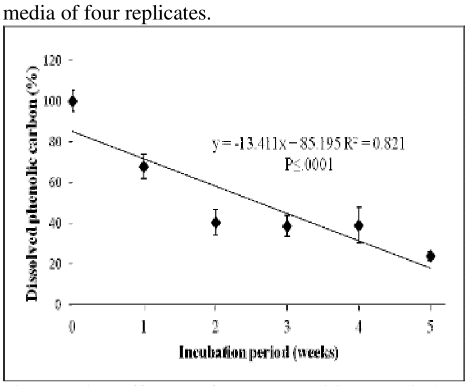

Figure 5. Effects of decomposition period of *P. javanica* on percent dissolved phenolic carbon. The error bars are standard deviations (SD) and the bars represent the media of four replicates.

P. javanica decreased the radicle length of *P. polystachion* by 43% and 14%, respectively. At 10 g L^{-1} *P. javanica* reduced the *P. polystachion* radicle length by 3%.

Shoot lengths of *A. gangetica* and *P. polystachion* increased with increasing *P. javanica* litter leachate concentration amended soil, but these increases were not significant (R^2= 0.02, P=0.57 and R^2= 0.75, P=0.12, respectively). The changes in *A. gangetica* and *P. polystachion* root lengths were not different with various litter leachate amended soils (R^2= 0.03, P=0.92 and R^2= 0.17, P=0.52, respectively). Seedling dry weights of *A. gangetica* and *P. polystachion* showed a linearly increasing trend with increasing leachate amended soils, but the differences were not significant (R^2= 0.25, P=0.16 and R^2= 0.30, P=0.53, respectively). The *P. javanica* leachates did not effect the total chlorophyll content in the leaves of *P. polystachion* (R^2= 0.15, P=0.234) and *A. gangetica* (R^2= 0.20, P= 0.323).

The release pattern and amounts of dissolved phenolic compounds released into the soil by the decomposing *P. javanica* litter is presented in Figure 5. The dissolved phenolics compounds contents in soil were determined to know changes in their contents over time. Their contents decreased during the 6-weeks decomposition period (Fig. 5). The dissolved phenolic compounds content after 1 week was 68%, which decreased to 40.5, 38.6, 39 and 23.9% at 2, 3, 4 and 5 weeks after incubation, respectively. This implies that phenolic compounds decreased by 32, 59, 60, 61 and 76% at 1, 2, 3, 4, and 5 weeks after incubation, respectively.

Water and acetone extractable phenolics of *P. javanica* shoots and litter showed that the level of water and acetone extractable phenolics in *P. javanica* shoots was higher than in litter. Water extractable phenolics from litter was 163 ppm and from the shoots was 687 ppm. The acetone extractable phenolics from the litter and shoots were 322 ppm and 1543 ppm, respectively.

DISCUSSION

The phytotoxicity of *P. javanica* litter was evaluated using an aqueous extract bioassay. This effect was investigated on the germination of seeds and growth of young seedlings of weeds. The germination and growth of young seedlings was studied because the seed germination is most important pivotal periods in seedling recruitment and population dynamics for seed plants (39). *Pueraria javanica* litter leachates reduced and delayed the germination of *A. gangetica*. Germination of *P. polystachion* was not affected by the aqueous litter leachates of *P. javanica*. However, the radicle lengths of both weed species were retarded. Similarly, the fresh aqueous shoot extracts of *Tithonia diversifolia* did not show allelopathic effects on the germination of *Zea mays*; however, the radicle and plumule lengths of the seedlings were significantly inhibited by the aqueous extract (27). There was a reducing trend in radicle growth with increasing extract concentration. These results suggest that seeds were able to germinate, but produced seedlings with impaired growth. The radicle growth inhibition started at the lowest applied concentration (10 g L^{-1}).

The phenolic acids concentrations of 100-1000 ppm are allelopathically active and toxic to seedlings (13,25,36). In the present study, the concentrations of phenolics in the *P. javanica* shoot and litter were >100 ppm, which suggests that the *P. javanica* had allelopathic potential. However, the two test species differed in their germination and growth sensitivity. The *P. javanica* extracts exhibited higher inhibition of *A. gangetica* germination than that observed for *P. polystachion*. However, the *P. javanica* extracts were more effective at inhibiting radicle growth of *P. polystachion* (grass weed) than *A. gangetica* (broad-leaved weed). In fact, the radicle growth reduction value of *P. polystachion* was 20% higher than *A. gangetica* at the 50 g L^{-1} concentration. This suggests that attributes of seed germination and seedling growth of weeds were differentially susceptible to aqueous extracts of cover crops (10,16). The different responses of bioassay species to cover crop litter extracts might be due to evolutionary differences in the resistance to allelopathic compounds among the target species. Qasem (29) showed that white top and Syrian sage are of great allelopathic potential against cabbage, onion, and tomato compared to carrot, cucumber, squash and pepper. Studies on allelopathic effects of leachates from *Ceratiola ericoides* on seven rosemary scrub species showed that the seven subordinate rosemary scrub species responded differently (14).

There were some attempts to assess the phytotoxicity of conventional legume cover crops. Shahid *et al*. (33) reported that the germination, radical length and dry weight of *A. intrusa* (*A. gangetica*) decreased when grown in full-strength aqueous extract (66.6 g L^{-1}) of *Calopogonium caeruleum,* but these characteristics were not affected by the presence of *Centrosema pubescens*. Arruda *et al*. (1) isolated three potential allelochemicals from *Pueraria phaseoloides* (*P. javanica*), which showed selective herbicidal activity against the tested weed species. The dichloromethanic and ethyl acetate fractions significantly infuenced the seed germination of *Mimosa pudica*, with 100 and 76% inhibition, respectively. Moreover, the three isolated compounds had greater effects on radicle elongation of *M. pudica*, *Senna obtusifolia*, *Senna occidentalis* and *Urena lobata*. Casini and Olivero (4) tested the influence of seed leachates, water extracts of residues and root exudates of legume cover crops *viz*., *Puereria phaseoloides*, *Canavalia ensiformis* and *Mucuna pruriens* on germination and seedling growth of *Imperata brasilinsis*. The water extracts of shoot residues of all cover crops promoted germination, while, the germination index was remarkably delayed by 22 and 26% with the highest extract concentration (4%) of *M. pruriens* and *Canavalia ensiformis*, respectively. The 54% concentrations of *Puararia javanica* leaf extract, reduced the germination and growth of *Borreria alata* by 50% and 60%, respectively.

The interactions of allelochemicals with soil components upon release from the plant is important in determining whether or not inhibition of the target plant is likely to occur in the field (2,15). As soil factors can modify allelochemicals and alter their chemistry (18), it is important to involve soil in bioassays to demonstrate allelopathy. In this study, the growth of two week old weeds sown in *P. javanica* litter leachate amended soils were observed to be similar to plants in the control treatment. The application of litter leachate amended soils did not reduce shoot length, root length, dry weight and total chlorophyll accumulation. These studies revealed that aqueous *P. javanica* litter extracts could result in different effects on seedling growth of test weeds, depending on planting media (with or without soil). Similarly, Ohno *et al*. (25) showed that clover residues significantly decreased radicle the growth of wild mustard by 20% at the first sampling after red clover incorporation (8 days after incorporation) and water soluble phenolic compounds were involved in the reduction of wild mustard root growth. Ohno *et al*. (25) believed that phenolic compounds released by the decomposing clover residues were adsorbed and/or oxidized by the soil and hence the adsorbed phenolics were found to be much greater than available soluble phenolics. In another study Ohno and Doolan (26) showed that in the absence of soil (adsorbents), the phenolic compounds from red clover decomposition were stable throughout the 5-week incubation, in contrast to the shorter period of toxicity in field soils (25). These results suggest that the adsorption processes is a key factor in determining the level of phytotoxcity observed after residue incorporation.

Many sustainable cropping systems include cover crops as green manures to supply N and reduce soil erosion. Recent work has shown that this practice may also contribute to weed management by altering the temporal dynamics of nutrient availability or by direct phytotoxic effects (20). Reduction in the growth of certain plants was correlated most strongly with the concentration of phenolic carbon in these cover crops, suggesting that the release of phenolics into the soil inhibits the growth of certain plants (3,29). These naturally active secondary metabolites can be released into the environment either as exudates from living plant tissues or after incorporation or desiccation of residue (28). Phenolic compounds are ubiquitous constituents of plant tissues and have been the focus of many allelopathy studies (15).

The litter incubation study in soil was conducted to gain insight into the fate of allelochemicals from *P. javanica* litter in a soil system after several weeks of incubation under simulated natural conditions. The phytotoxic activity of allelochemicals in soil is a function of complex interactions among soil and plant factors. From an allelopathic perspective, phytotoxic compounds are not considered suitable if they are not released into their environment, and the fate of the allelochemical in the soil should be considered. Once an allelochemical or a mixture of allelochemicals enters to a soil system, processes such as adsorption–desorption, microbial decomposition and leaching can modify its behavior (18).

Several studies have proposed techniques to extract the allelochemicals from soil. However, Whitehead *et al.* (36) concluded that phenolics extracted with water were ecologically more important. Hence, in this study only the water-soluble fractions of phenolics in the soil were considered. The results showed that the dissolved phenolics tended to decrease during the 6 week decomposition period. The phenolic content in the soil, which was one of the degradation products of litter decomposition, was higher in the initial period (0 week), but after one week of incubation, the amount of soluble phenolics declined abruptly and then leveled off over time. The concentration of soluble phenolics in the soil was 68% after the first week of incubation and 24% after the sixth week. Other authors have also reported the fast disappearance of phenolics within the first week or month. Shofield *et al.* (34) reported the disappearance of more than 50% of phenolics from willow leaves within 2 weeks of incubation. Rashid *et al.* (30) reported 69% soluble phenolics in the soil after the first week of incubation of kudzu litter and a 62% phenolic content after the sixth week. The reduction in phenolic compounds over the five-week period suggest that microbes were utilizing the phenolic compounds during the incubation period (26). Ohno and Doolan (26) and Rashid *et al.* (31) reported that the trend in dissolved organic carbon concentrations in the soil is comparable to that of soluble phenolics. However, in this study it appears that soil and environment condition were responsible for the fast decomposition of dissolved organic carbon and changes in soluble phenolics.

CONCLUSIONS

The results of bioassay, growth and litter incubation studies concluded that *P. javanica* litter interferes allelopathically with its neighboring species. However, the allelopathic effects of litter on germination depend on weed species. Interactions among soil and plant factors influences the phytotoxic effects of allelopathic compounds on seedling growth. It was apparent that the allelopathic action did not persist for considerable periods of time.

REFERENCES

1. Arruda, M.S.P., De Araújo, M.Q., Lôbo, L.T., De Souza Filho, A.P., Alves, S.M., Santos, L.D.S., Müller, A.H. and Guilhon, G.M.S.P. (2005). Potential allelochemicals isolated from *Pueraria phaseoloides*. *Allelopathy Journal* **15:** 211- 220.
2. Blum, U. (1995). The value of model plant-microbe-soil systems for understanding processes associated with allelopathic interactions. In: *Allelopathy: Organisms, Processes and Applications* (Ed., Inderjit, K.M.M. Dakshini and F.A. Einhellig), *ACS Symposium Series* **582**: 127- 131. American Chemical Society, Washington, DC.
3. Blum, U. (2011). *Plant–Plant Allelopathic Interactions: Phenolic Acids, Cover Crops and Weed Emergence*. Springer, London, New York.
4. Casini, P. and Olivero, L. (2001). Allelopathic effects of legume cover crops on cogon grass (*Imperata brasiliensis* Trin.). *Allelopathy Journal* **8:** 189- 200.
5. Chew, P.S., Kee, K.K. and Goh, K.J. (1999). Cultural practices and their impact. In: *Oil Palm and the Environment- A Malaysian Prespective* (Eds., S. Guemit, K.H. Lim, L. Teo and D.K. Low), pp. 55- 80. Malaysian Oil Palm Growers' Council, Kuala Lumpur.
6. Chung, I.M., Ahn, J.K. and Yun, S.J. (2001). Identification of allelopathic compounds from rice (*Oryza sativa* L.) straw and their biological activity. *Canadian Journal of Plant Science* **81**: 815- 819.
7. Dakshini, K.M.M., Foy, C.L. and Inderjit, S. (1999). Allelopathy: One component in a multifaceted approach to ecology. In: *Principles and Practices in Plant Ecology, Allelochemical Interactions* (Ed., S. Inderjit, C.L. Foy and K.M.M., Dakshini), pp. 3- 14. CRC Press. Boca Raton, Florida, USA.
8. Dalton, B.R. (1999). The occurrence behavior of plant phenolic acids in soil environments and their potential involvement in allelochemical interference interactions: Methodological limitations in establishing conclusive proof of allelopathy. In: *Principles and Practices in Plant Ecology: Allelochemical Interactions* (Ed., S. Inderjit, K.M.M. Dakshini and C.L. Foy). CRC Press, Boca Raton, Florida, USA.
9. Dalton, B.R., Blum, U. and Weed, S.B. (1989). Differential sorption of exogenously applied ferulic p-courmaric p-hydroxybenzoic and vanillic acids in soil. *Soil Science Society of America Journal* **53:** 757- 762.
10. El-darier, S.M. and Youssef, R.S. (2000). Effect of soil type, salinity, and allelochemicals on germination and seedling growth of a medical plant *Lepidium sativum* L. *Annals of Applied Biology* **136:** 273- 279.
11. FAO. (2012). Ecocrop: *Pueraria phaseoloides*. from http://ecocrop.fao.org/ecocrop/srv/en/ cropView?id =1824
12. Fujii, Y., Shibuya, T. and Usami, Y. (1991). Allelopathic effect of *Mucuna pruriens* on the appearance of weeds. *Weed Research,* Japan **36:** 43- 49. (Japanese with English summary).
13. Guenzi, W.D. and McCalla, T.M. (1962). Inhibition of germination and seedling development by crop residues. *Soil Science Society of America Proc*eeding **26:** 456- 458.
14. Hunter, M.E. and Mengers, E.S. (2002). Allelopathic effects and root distribution of *Ceratiola ericoldes* (*Empetraceae*) on seven rosemary scrub species. *American Journal of Botany* **89:** 1113- 1118.
15. Inderjit, K. (1996). Plant phenolics in allelopathy. *Botanical Review* **62:** 186- 202.
16. Jefferson, L.V. and Pennacchio, M. (2003). Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. *Journal of Arid Environments* **55:** 275- 285.
17. Kamo, T., Hiradate, S. and Fujii, Y. (2003). First isolation of natural cyanamide as a possible allelochemical from hairy vetch (*Vicia vilosa*). *Journal of Chemical Ecology* **29:** 275- 283.
18. Kobayashi, K. (2004). Factors affecting phytotoxic activity of allelochemicals in soil. *Weed Biology and Management* **4:** 1- 7.

19. Lehmann, R.G., Cheng, H.H. and Harsh, J.B. (1987). Oxidation of phenolic acids by soil iron and manganese oxides. *Soil Science Society of America Journal* **51:** 352- 356.
20. Liebman, M. and Ohno, T. (1997). Crop rotation and legume residue effects on weed emergence and growth: Application for weed management. In: *Integrated Weed and Soil Management* (Ed., J.L. Hatfield, D.D. Buhler and B.A. Stewart), pp. 181- 221. Ann Arbor Press, Chelsea, MI.
21. Lottina-Hennsen, B., King-Diaz, B., Aguilar, M.I. and Fernandez-Terrones, M.G. (2006). Plant secondary metabolities, targets and mechanisms of allelopathy. In: *Allelopathy: A Physiological Process with Ecological Implications* (Ed., M.J. Reigosa, N. Pedrol and L. González). Springer, Dordrecht, the Netherlands.
22. Makino, T., Takahashi, Y., Sakurai, Y. and Nanzyo, M. (1996). Influence of soil chemical properties on adsorption and oxidation phenolic acids in soil suspension. *Soil Science and Plant Nutrition* **42:** 867- 879.
23. Morgan, E.C. and Overholt, W.A. (2005). Potential allelopathic effects of Brazilian pepper (*Schinus terebinthifolius* Raddi, Anacardiaceae) aqueous extract on germination and growth of selected Florida native plants. *The Journal of the Torrey Botanical Society* **132**:11- 15.
24. Murtini, I., Fatonah, S. and Izda, M.N. (2013). Potensi alelopati ekstrak daun *Pueraria javanica* terhadap perkecambahan dan pertumbuhan anakan gulma *Borreria alata* (Aublet) DC. From http://repository.unri.ac.id: 80/handle/123456789/2402.
25. Ohno, T., Doolan, K., Zibilske, L.M., Liebman, M., Gallandt, E.R. and Berube, C. (2000). Phytotoxic effects of red clover amended soils on wild mustard seedling growth. *Agriculture, Ecosystems and Environment* **78:** 187- 192.
26. Ohno, T. and Doolan, K.L. (2001). Effects of red clover decomposition on phytotoxicity to wild mustard seedling growth. *Applied Soil Ecology* **16:** 187- 192.
27. Oyerinde, R.O., Otusanya, O.O. and Akpor, O.B. (2009). Allelopathic effect of *Tithonia diversifolia* on the germination, growth and chlorophyll contents of maize (*Zea mays* L.). *Scientific Research and Essays* **4:** 1553- 1558.
28. Putnam, A.R. and Tang, C.S. (1986). *The Science of Allelopathy*. Wiley, New York.
29. Qasem, J.R. (2001). Allelopathic potential of white top and syrian sage on vegetable crops. *Agronomy Journal* **93:** 64- 71.
30. Rashid, M.H., Asaeda, T. and Uddin, M.N. (2010a). The Allelopathic Potential of Kudzu (*Pueraria montana*). *Weed Science* **58:** 47- 55.
31. Rashid, M.H., Takashi, A. and Uddin, M.N. (2010b). Litter-mediated allelopathic effects of kudzu (*Pueraria montana*) on *Bidens pilosa* and *Lolium perenne* and its persistence in soil. *Weed Biology and Management* **10**: 48- 56.
32. Ruan, S., Xue, Q. and Thlkowska, K. (2002). Effect of seed priming on germination and health of rice (*Oryza sativa* L.) seeds. *Seed Science and Technology* **30:** 451- 458.
33. Sahid, I., Tasrif, A. and Latiff, A. (1993). Allelopathic potential of legume cover crops on selected weed species. *Plant Protection Quarterly* **8:** 49- 53.
34. Shofield, J.A., Hagerman, A.E. and Harold, A. (1998). Loss of tannin and other phenolics from willow leaf litter. *Journal of Chemical Ecology* **24:** 1409- 1421.
35. Weir, T.L., Park, S.W. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Current Opinion in Plant Biology* **7:** 472- 479.
36. Whitehead, D.C., Dibb, H. and Hartley, R.D. (1981). Extractant pH and the release of phenolic compounds from soils, plant roots, and leaf litter. *Soil Biology and Biochemistry* **13:** 343- 348.
37. Wibawa, W., Mohamad, R., Juraimi, A.S., Omar, D., Ghazali Mohayidin, M. and Begum, M. (2009). Weed control efficacy and short term weed dynamic impact of three Non-selective herbicides in immature oil palm plantation. *International Journal of Agriculture and Biology* **11:** 145- 150.
38. Witham, F.H., Blaydes, D.F. and Devlin, R.M. (1986). *Exercises in Plant Physiology*. PWS Publishers, Boston, USA.
39. Xing, F., Guo, J.X. and Wang, Y.H. (2003). Seed germination characteristics and regeneration mechanism of *Stellera chamaejasme* population. *Chinese Journal of Applied Ecology* **14:** 1851- 1854.
40. Xuan, T.D., Shinkichi, T., Khanh, T.D. and Min, C.I. (2005). Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: An overview. *Crop Protection* **24**: 197- 206.
41. Zimdahl, R.L. (2007). *Fundamentals of Weed Science*. Elsevier Inc. Amsterdam, Nethelands.