

## SHORT COMMUNICATION

### Effects of *Mikania micrantha* extracts and their exposure time on seed vigour, seed germination and seedling growth of plants

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#### ABSTRACT

We studied the allelopathic effects of *Mikania micrantha* on seed vigour, seed germination and seedling growth of three receptor species (*Robinia pseudoacacia* L., *Lolium perenne* L. and *Lagerstroemia indica* L.) under different exposure times. The target species were selected from the *Mikania*-invaded ecosystem in South China. The inhibitory effects on seed vigour, seed germination and seedling growth of these test species increased with increasing exposure times and at higher extract concentrations. Seed vigour was more sensitive to extracts of *Mikania* than seed germination and seedling growth. *Robinia pseudoacacia* was more resistant to *Mikania* extract than *Lolium perenne* and *Lagerstroemia indica*. We found that even short exposure time (50-100 h) to *Mikania* extract had strong allelopathic effects on seed vigour, seed germination and seedling growth of test species.

**Key Words:** Allelopathy, exposure time, *Lagerstroemia indica*, *Lolium perenne*, *Mikania micrantha*, *Robinia pseudoacacia*, seed germination, seed vigour, seedling growth.

#### INTRODUCTION

The invasive vine weed (*Mikania micrantha*), has caused major ecological and economical problems and great damage to native plant communities in natural and agricultural lands in South China (32). Besides competing for growth resources with other plants, it also inhibits the seed germination and seedling growth of co-occurring plants (18,20,33). Allelopathy plays an important role in invasion success of some exotic plants (8,27,32). Although the role of allelopathy has been proved in the field, but its mechanism is difficult to demonstrate without controlled experiments, due to difficulty in separating resource competition from allelopathy (2). Allelopathic effects have been shown on seed germination and seedling growth in Petri dish bioassays, greenhouse experiments or controlled field experiments (9-10). During early stages of imbibition, under allelopathy stress low vigour seeds decreases the membrane integrity and thus more cytoplasmic solutes are released into the imbibing medium than from high vigour seeds (29). Their seed vigour can be determined by measuring the conductivity of their seed leachates (29). The

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seed vigour is more sensitive than seed germination to test seed quality, hence, it is used to test the allelopathic effects on seed germination and seedling growth (6,11,21,34). Aqueous extracts are most frequently used, because most natural allelochemicals from plants' aboveground tissues are leached into soil by water. The aqueous extracts are prepared from different tissues or organs (or mixtures of plant tissues or organs), different residues, different ages and various concentrations (9-10,18,27-28,35), but the effects of different exposure times have not been determined. As the coastal regions in Guangdong Province, China, suffer from some extreme natural phenomenon (storms, floods and typhoons etc.) and disturbance from anthropogenic activities. Thus some seeds change their locations, due to secondary seed dispersal (31). Therefore, such seeds may encounter different periods of exposure to allelochemicals released from the invasive species such as *Mikania micrantha*. However, the allelopathic effects on such seeds have not been studied.

To test the allelochemical effects of *Mikania*, we selected 3 test species (*Robinia pseudoacacia* L., *Lolium perenne* L. and *Lagerstroemia indica* L.) from the *Mikania*-invaded ecosystem in South China. We hypothesized that the allelopathic effects of *M. micrantha* increased on seed germination and seedling growth of these target species with exposure time, furthermore, the allelopathic effects on seed vigour also increased with both exposure times and extract concentrations.

## MATERIALS AND METHODS

Three kg *M. micrantha* plants were collected from Shenzhen University (114° 04' E, 22° 37' N, 62 m asl.) in July 2007 and separated them into leaves and roots. These parts were air-dried and milled into fine powder with a mortar and a pestle. Fifty g of each fine powder was soaked for 24 h in 1 L distilled water at room temperature ( $25 \pm 3$  °C) and shaken. The extract (5%, w/v) was filtered through four layers of cheese cloth to remove the solid materials. The pH of aqueous extract was adjusted to 7.0 with 1 M NaOH or HCl. This aqueous extract of ( $50 \text{ g L}^{-1}$ ) concentration was kept at 4 °C till use.

Seeds of *Lolium perenne* L. were purchased from the Chinese Academy of Forestry Sciences and those of *Lagerstroemia indica* L. and *Robinia pseudoacacia* L. from the China National Trees Seeds Corporation. The seeds of *M. micrantha* were collected from the South China in 2006 and kept at 4 °C until use. All seeds were surface sterilized with 5% hydrogen peroxide for 20 min and then rinsed several time with distilled water.

### Duration of exposure

Though 5% (w/v) extracts are commonly used (1,7,22), but this concentration is considered higher than in nature (1). We conducted a preliminary experiment to test whether the negative effects of *Mikania* aqueous extract on seed vigour, seed germination and seedling growth were due to osmotic potential or allelochemicals. In the preliminary experiment, we used four concentrations, (0, 0.06, 0.6, and 6.0%) of polyethylene glycol (PEG-8000, Sigma), to determine the possible osmotic effects of extracts. This experiment proved that the negative effects were not caused by osmotic potential but by allelochemicals, which agrees with results of Ismail and Chong (18).

The experimental treatments consisted of three factors: 2 *M. micrantha* extracts (root and leaf), 3 target species (*Robinia pseudoacacia*, *Lolium perenne* and *Lagerstroemia*

*indica*) and 5 exposure times: [0 (control), 1, 10, 100 and >100 h]. Ten seeds of each species were placed in 9-cm Petri dish; one ml of distilled water was added to keep the seeds moist before applying the treatments and treatments were replicated 8 times. The extracts (distilled water for control) to 0 h, 100 h and >100 h were added to Petri plates, immediately, while the extracts to 10 h and 1 h treatments were added after keeping Petri dishes at 4 °C for 90 h and 99 h, respectively. All these Petri plates were kept at 4 °C for 100 h to prevent the seeds germination and keep uniform soaking time. Afterwards the seeds of each > 100 h treatment were directly transferred to new Petri dish (lined with two filter papers) and 5 ml extract was added as per treatment. While the seeds of other treatments were rinsed with distilled water as quickly as possible and placed in a new 9-cm Petri dish (lined with two filter paper) and then 5 ml distilled water was added. The covered Petri dishes were then incubated in incubator at 14 h light (28 °C) and 10 h in darkness (20 °C) and 75% relative humidity. Seeds were considered germinated, when the radicle length was > 2 mm and the number of germinated seeds were counted daily. The day the first seed germinated in each dish was considered as the initial germination time (IGT). Germination rate (GR) was calculated as under:

$$GR = (N_1 \times 1) + (N_2 - N_1) \times 1/2 + (N_3 - N_2) \times 1/3 + \dots + (N_n - N_{n-1}) \times 1/n,$$

Where,  $N_n$ : Number of germinated seeds obtained on the first (1), second (2), third (3), ..., (n-1), and (n) days.

The experiment was terminated, when no seed germinated in 3-days. The root length (RL) and shoot height (SL) of 24 seedlings per treatment were recorded at day 7, 7 and 20 for *R. pseudoacacia*, *L. perenne*, *L. indica*, respectively. All data was expressed as a percentage of control.

#### Effects of extract concentrations and exposure time

Only leaf aqueous extracts (pH adjusted to 7.0) were used in this experiment and only two lower concentrations (0.5% and 0.05%) were used in this experiment, as 5% (w/v) extract is considered higher than nature (1). The 5%, 0.5% and 0.05% extracts were ranked high, intermediate and low concentrations, respectively. All aqueous extracts were stored at 4 °C until use. Thirty seeds of each specie were placed in a separate 9-cm Petri dish and 5 ml extract (distilled water for control) was added as per treatments, then these were kept in dark at 4 °C (to prevent germination). As seed germination and seedling growth were negatively affected at 100 h exposure time in previous experiment, only 3-exposure times (control-0 h, 50 h and 100 h) were used and treatments were replicated 6 times. After treating for due time, seeds of every dish were rinsed several times with de-ionized water ( $0.058 \pm 0.012 \mu\text{s cm}^{-1}$ ) and transferred to 100 ml plastic square bottle (cleaned with de-ionized water) and 25 ml de-ionized water was added and kept in a incubator at 20 °C in dark for 24 h. After shaking several times, 1 ml solution in each bottle was diluted to 50 ml and its electrical conductivity (EC) was measured with conductivity meter (HANNA HI98309, Italy).

**Statistical analysis:** Three-way analysis of variance (ANOVA) was used to test the effect of target species, exposure time and concentrations of leaf extracts of *Mikania* on seed germination, seedling growth and electrical conductivity (SPSS 13.0, SPSS Inc., Chicago) and contrast analysis for non-lasting treatments was conducted at the same time. Homogeneity of variances was tested by using Levene's test and the least significant differences (LSD) between means were determined at 95% confidence level.

## RESULTS AND DISCUSSION

The *Mikania* leaf and root extracts significantly affected the seed germination (%), initial germination time and germination rate and seedling growth (root length and shoot height) of test species and the inhibition degree increased with exposure time (Figs. 1, 2).

*R. pseudoacacia* was more resistant to *Mikania* extracts than other two species (Figs. 1, 2). Leaf extract of *Mikania* was more inhibitory than root extract. Lasting treatments decreased the seed germination and seedling growth of target species; but non-lasting treatments although did not stimulate the seed germination, but promoted their seedlings growth (Figs. 1, 2). The non-lasting treatments ( $\leq 100$  h exposure time) inhibited the seed germination (except initial germination time) and stimulated the seedling growth.

Compared to control, leaf extract of *Mikania* significantly enhanced the electrical conductivity of three target species and the increment was increased with exposure time and concentration (Fig. 3).

The reactions of electrical conductivity varied among the bioassay species. *L. indica* was most sensitive, *R. pseudoacacia* was most resistant and *L. perenne* was intermediate and interactions were not found among species, concentration and exposure time (Fig. 3).

Negative effects of aqueous extracts of *Mikania* on seed vigour, seed germination and seedlings of the test plants were due to the allelochemicals present, but were not caused by osmotic potential, which agrees with Ismail and Chong (18). Because many allelochemicals are present in *Mikania* extracts (4,16,18, 26). As expected, extracts of *Mikania* adversely affected the seed vigour, seed germination and seedling growth of all test species and the inhibition degree increased with exposure time, which is first report in our study. Thus allelopathic effects of *Mikania* on seed vigour, seed germination and seedling growth of target species were correlated with the exposure time. In our experiment, short exposure time ( $\leq 100$  h) with high extract concentration may promote or inhibit seed germination and seedling growth as reported earlier (18), which suggested that stimulatory and inhibitory effects are functions of extract concentration and exposure time. Non-lasting exposure time could affect the seed germination and seedling growth; i.e. allelopathic effect continued if the seed was exposed to *Mikania* extracts for enough time.

In agreement with former studies, the allelopathic effects on seed vigour of test species increased with extract concentration (6,11,21,34). If only the seed germination and seedling growth of receptor plants are inhibited, it indicates that the receptor plants suffered from the allelopathic stress as observed in our experiment (15,19,25). Under allelopathic stress, seeds and seedlings of target species were exposed to oxidative reactions, if the antioxidant system could not cope with the rise of reactive oxygen species (ROS), then direct attack of ROS on cell membrane would cause lipid

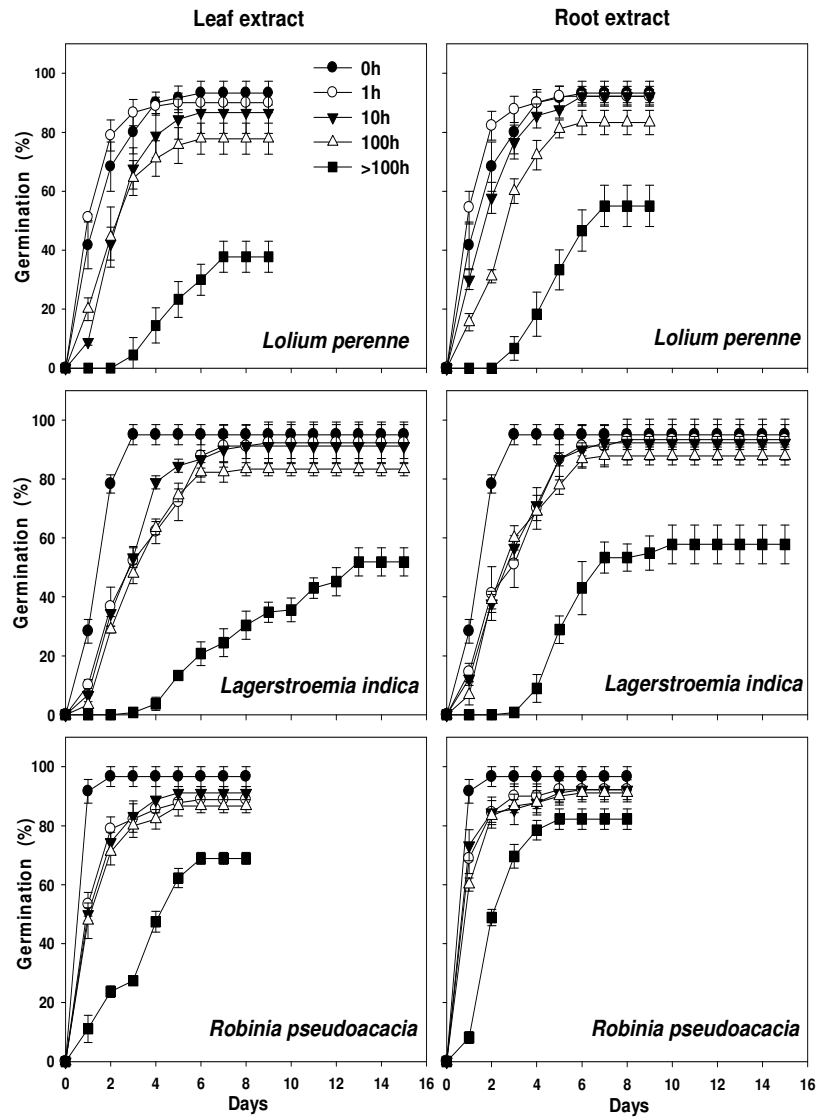


Figure 1. The allelopathic effects of *Mikania* leaf and root extracts on seed germination of 3 test species (*Robinia pseudoacacia*, *Lolium perenne* and *Lagerstroemia indica*) at various exposure times (0h, 1h, 10h, 100h and >100h). Bars represent standard errors.

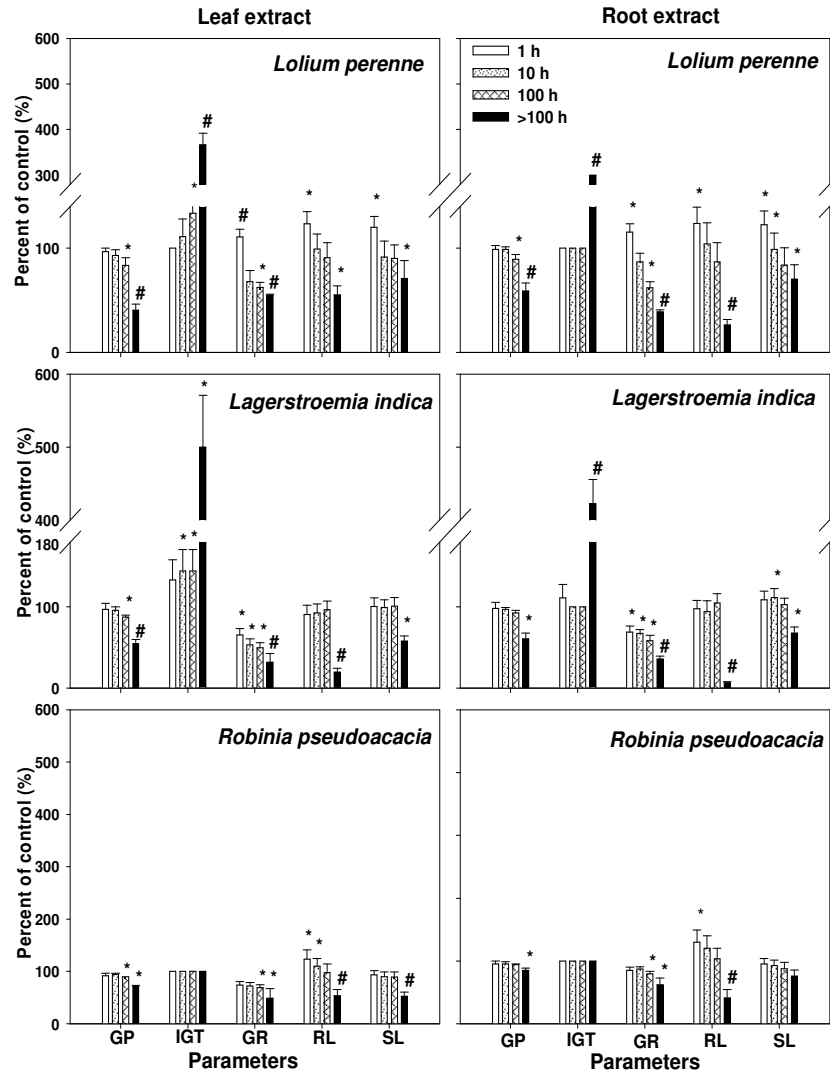


Figure 2. The allelopathic effects of *Mikania* leaf and root extracts on seed germination percent (GP), initial germination times (IGT), germination rate (GR), seedlings root length (RL) and shoot height (SL) of the 3 target species (*Robinia pseudoacacia*, *Lolium perenne* and *Lagerstroemia indica*) at four exposure times (1h, 10h, 100h and >100h) over the control. Bars represent standard errors; \*, p<0.05; #, p<0.01.

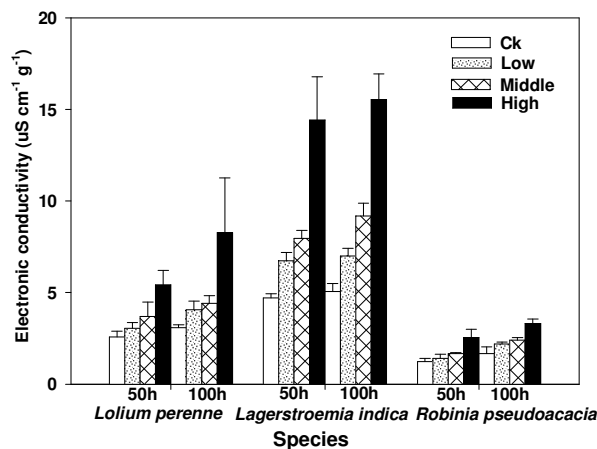


Figure 3. The allelopathic effects of leaf extract of *Mikania* at two exposure times (50 and 100 h) on the electric conductivity of three target species (*Robinia pseudoacacia*, *Lolium perenne* and *Lagerstroemia indica*). Bars represent standard errors; Bars labeled with different letters represent significantly different ( $p < 0.05$ ; LSD test)

peroxidation (LPO), and may also even result in breakdown of permeability in cell membrane and cell death (15). Our experiment verified the trend, because seeds treatment with *Mikania* extract resulted in rapid and marked electrolyte leakage, which shows the damage to plasma membrane and the cellular damage decreased the germinability and gradual loss of seed vigour as found in our experiment (34). The three target species showed different responses to *Mikania* extract, which is common phenomenon in allelopathy (12,17), because they have different evolutionary history and varying resistance to allelochemicals (17,24). *R. pseudoacacia* was more resistant to allelopathy of *Mikania*, because of its bigger seeds than other two species, thus, it has more nutrition (energy) to resist allelopathic effects (23,30).

The leaf extract showed stronger allelopathic effects than root extract on seed germination and seedlings of target species, which agrees with previous studies (10,27-28,30). It may be owing to differences in concentrations of diffusible allelochemicals and chemical composition (5,10). To clarify such differences, we should isolate and identify the chemicals in leaf and root extracts.

In our study, the allelopathic effects not only decreased the germination but also delayed the germination, (13). As the seedling survival depends on seed size, the delayed seedlings could hardly compete in water-stress environment, if their emergence is retarded (14). Therefore, low germination rate and delayed germination due to allelopathic effects of *Mikania* would put the target species in disadvantage for restoration in invasive ecosystems (3).

Based on these and previous similar studies (31), our study verified the strong allelopathic potential of *Mikania*, which decreased the seed vigour, seed germination and seedling growth of target species even with a short exposure time, but more experiments using soils or in field experiment are needed.

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