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Allelopathic effects of decomposing leaf litter of camphor (*Cinnamomum camphora* (L.) Presl) on harvested seeds germination and seedlings growth of balsamine (*Impatiens balsamina* L.) and morning glory (*Ipomoea nil* (L.) Roth)

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ABSTRACT

In pot culture, we investigated the effects of decomposing litter of camphor [*Cinnamomum camphora* (L). Presl] on the growth of companion plants [Balsamine (*Impatiens balsamina* L) and Morning glory (*Ipomoea nil* L)] focusing on flowering traits, harvested seed quality, seed germination and seedlings growth in soil incorporated with/without the leaf litter. The camphor leaf litter dose of 0.56 % (mass fraction) significantly inhibited the vegetative growth indices (plant height, root collar, biomass and leaf development) of both receiver plants. Both recipient plant species showed delayed first-flower day, lower flowering velocity and decreased flower numbers after exposure to the leaf litter. Morning glory bloomed with smaller flowers at the higher (11^{th}~15^{th}) nodes. Despite these negative effects, the 1000-grain weight and germination of the seeds produced by the two recipient plants and growth performance of their offspring were slightly impacted. The balsamine was more sensitive than morning glory. By GC-MS, we detected 3- terpenoid compounds (Camphor, 1,8-Cineole and *β*-Caryophyllene), these caused allelopathic effects during camphor leaf litter decomposition.

Key words: Allelopathy, balsamine, camphor tree, *Cinnamomum camphora*, decomposition, essential oil, *Impatiens balsamina*, *Ipomoea nil*, leaf litter, morning glory, reproductive growth, seed quality, seedlings growth, terpenoids

INTRODUCTION

Cinnamomum species (family Lauraceae), are generally evergreen and aromatic. They are naturally distributed in Asian countries featuring tropical or subtropical climate and in Pacific islands, or artificially planted to serve road and garden landscaping, or to provide timber, essential oils, spice and medicine (34). Also, they are newly tried to be applied in nanoparticle synthesis (46) and remediation of heavy metal contaminated environments (15).

However, the ecological impacts of *Cinnamomum* trees are related to its allelopathy. Turf grass degenerated seriously after sowing into a camphor [*C. camphora* (L.) Presl] stand (41). Specialized plant communities of *C. longe paniculatum* (Gamble) N. Chao ex H. W. Li forests caused the abnormal growth of adjacent crops. The water extract of its stem and leaves suppressed the germination of wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) (31) and interfered with the mitosis of the root tip cells of broad bean

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(*Vicia faba* L.) (11). Leachate of rhizosphere soil of camphor affected the germination and seedling growth of three vegetable crops (26). Aqueous extracts of *C. septentrionale* Hand.Mazz. leaf litter were detrimental to photosynthesis and growth of *Eucalyptus grandis* Hill ex Maiden saplings (16). The essential oils from camphor inhibited seed germination of wheat (30). Growth of rice seedlings was inhibited in soil incorporated with the leaf powder of camphor and in sea sand applied with the soil water from the leaf powder-incorporated soil (32). Suppressed growth and disturbed physiology were found in several plant species grown in soil infested by leaf litter debris of *C. septentrionale* (17,45) or *C. japonicum* Sieb. (3,39) There are various kinds of secondary metabolites in *Cinnamomum*, like phenols, esters, ketone and hydrocarbons (43,44), but more important and more frequently reported type are terpenoid compounds in their essential oils (20,33,38,40), they or some specific components have been bioassayed to account for allelopathic effects (13,32,35,36).

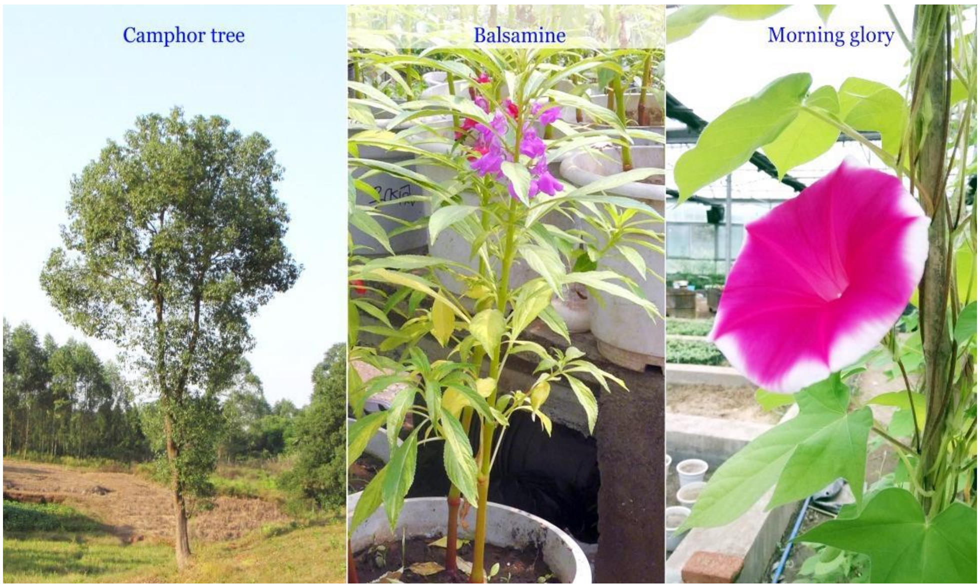

Figure 1. Donor plant and Recipient plants: *C. camphora,* balsamine and morning glory.

As a representative *Cinnamomum* species native to China, the defoliation of camphor is large (6000~8000kg/ha in a 30-yr old stand) and done during April -May in Sichuan Province of China. Its leaf litter thus, has great possibility to deliver bioactive substances and influence the growth of coexisting plants via decomposition. We have previously revealed that the decaying leaf litter of camphor greatly influenced the growth of receiver plants [*Brassica rapa* L. var. *chinensis* (L.) Kitamura, *Capsicum annuum* L., *Lactuca sativa* L., etc.], via inhibition of photosynthesis, induction of reactive oxygen species, and limitation of soil available nitrogen (8,29,47,48), and have identified the potential allelopathic components in the essential oil of leaf litter (5). But, the effects on reproductive growth and harvested seeds, are not clear yet.

In the present study, two graceful flowering plants widely used in landscaping and traditionally used as Chinese medicines, balsamine (*Impatiens balsamina* L.) and morning glory (*Ipomoea nil* (L.) Roth), were selected as recipients, considering their short lifecycle and apparent reproductive growth. We aimed to elucidate the allelopathic effects of decomposing litters of camphor on plant reproduction and succession, and verify the potential allelochemical(s) further. It was hypothesized that (i). vegetative and reproductive growth of the recipient plants are significantly affected, (ii).and harvested seed quality and their seedlings growth are also affected. We propose that this work can provide some guidelines for litter management of the ecosystems containing *Cinnamomum* tree species.

MATERIALS AND METHODS

Pot experiments were done in Teaching and Research Base, Sichuan Agricultural University, Ya'an, China (29°58′48″N, 102°59′55″E, altitude 600 m). It has subtropical humid monsoon climate. Annual average temp: 14.1 °C ~17.9 °C, Maximum temp: 29.9 °C (July), Minimum temp: 3.7 °C (January), Annual sunshine hours: 1039.6 h, frost-free period: 298 days, annual average rainfall: 1774.3 mm, and average air humidity: 79%.

Materials

Newly-fallen leaf litter of *C. camphora* (camphor tree) were collected in April from a 30-year-old forest in our University. The leaf litter was air-dried, cut into pieces (1 to 2 cms) for thorough mixing with soil**.** The recipients were two herbaceous ornamental plants [*Impatiens balsamina* (Balsamine) and *Ipomoea nil* (Morning glory)]. Their seeds were collected in the campus. The pot soil was sandy loam, collected from local farm. After air-drying, it was passed through sieve (1cm × 1cm mesh size) to remove stones and plant residues. Prior to use, the soil was mixed completely and 9 kg dry soil was placed in each plastic pot (top dia 29 cm, bottom dia 25 cm, and depth 26 cm)

POT EXPERIMENTS

To test of the effects of decomposing leaf litter of *C. camphora* on both receiver plants (Balsamine, morning glory), pot experiments were done from April to August 2013, as per method reported earlier (2,17). Briefly, 50 g leaf litter was added per pot (9 kg soil, biomass fraction 0.56%, equal to 7.57 t/ha) to simulate the natural decomposition. The leaf litter dose was based on the annual defoliation quantity (6-8 t/ha, i.e. 40~53 g per pot) of 30-year-old camphor tree forest. The leaf litter was not added in control pot. The experimental treatments consisted of two factors (i). Leaf litter doses 2 (0, 50g/ pot) and (ii). Recipient plants 2 (Balsamine, Morning glory). The treatments were replicated 5 -times in randomised block design.

Prior to seeds sowing, each pot was watered with 1.0 L tap water to wet the soil. On April 25th, thirty seeds of either Balsamine or Morning Glory were sown per pot on the surface and covered with a 1 cm-thick soil layer. Then, additional 500 mL tap water was slowly added. After seeds sowing, sufficient water was added to each pot every one or two days to ensure higher germination. During the experimental period 18 % soil moisture was maintained in pots.

The seedlings (Balsamine 64 days old, Morning glory 55 days old) were thinned to 2-plants per pot. Parameters of vegetative growth (height, root collar, biomass, leaf number and area) and reproductive growth (flowering dynamics and corolla size) of recipient plants were determined non-periodically. The mature fruits of Morning glory were harvested and labelled by the node position and the seeds were removed. The seeds of Balsamine plants were collected from its mature fruits. These seeds were sun-dried, weighed and stored in dry cool condition to determine their germination and seedlings growth in the next year.

(i). Height, root collar, biomass and leaf area: The seedlings height (measured with ruler), root collar (measured with electronic digital calliper), biomass weight (determined with electronic balance) and leaf number were determined at different growth stages. The cotyledon area and the first europhylla area were measured by coordinate grid method within 30 days after sowing. Briefly, a leaf is laid flat on a grid paper (Grid size 1 mm x 1 mm), and by counting the leaf margin surrounded grids, its area was obtained.

(ii). Flowering modelling: The corolla of morning glory is funnel-shaped and its size was measured as the mouth diameter, for each node along the stem. The number of flowers on each plant were counted daily from the beginning of flowering till the frutescence stage. Based on scatter plots with the accumulative flower numbers as the Y-axis and the leaf litter decomposition time as the X-axis, the logistic growth parabola was derived to describe the flowering dynamics. The following mathematic models (A) and (B) were used to model flowering in Balsamine and Morning glory, respectively.

$$Y=a/((1+b\cdot e^{cX})) \quad (A)$$
$$Y=a\cdot(1-e^{(-cX)})^{b} \quad (B)$$

Where, a, b, c are constants, and e is the natural log

The maximum accumulative flower number per plant (AF_{max}) was obtained from this model, and the other flowering parameters [the maximum flowering velocity (FV_{max}), the day the first flower appeared (D_{first}) and the day the last flower appeared (D_{last}), etc.] from the first-order derivative of the model.

(iii). Harvested seeds germination and seedling growth

To determine the vitality of harvested seeds, their germination and seedlings growth were studied in pot culture (soil substrate without leaf litter). Twenty seeds were sown per pot on April 17th. The 43 days old seedlings were thinned to 4- plants (Balsamine) and 3-plants (Morning glory) per pot. The seeds germination (%) was calculated two weeks after sowing to ensure that all viable seeds have emerged and seedlings morphological parameters (height and root collar) were measured till 63 days.

ESSENTIAL OIL

The essential oil from camphor leaf litter was previously analyzed to identify the insecticidal, antibacterial and antifungal substances (30,33,40). The analysis of potential allelochemicals has been done in 2012 using two samples, (i). the original camphor tree leaf litter and (ii). the decomposed leaf litter buried in soil for 4.5-month (5). In 2013 also, the essential oil of camphor tree leaf litter was analysed as per the method of Huang *et al.* (17) to know the variations in essential oil between two years.

Essential oils Extraction from leaf: Twenty g air-dried powdered leaf litter sample was mixed with 40 mL n-hexane and sonicated under 40 KHz at room temperature for 10 min, using a ultrasonicator (Skymen, China) and filtered through a quantitative filter paper (Jiaojie No.202, medium speed, maximum aperture 15~20 μm)**.** An additional 20 mL n-hexane was added and the powdered leaf litter was re-sonicated for 20 min and filtered again. The two filtrates were combined and transferred to rotary evaporators and concentrated. The concentrated solution was suspended in 5 mL n-hexane and filtered using a disposable Millex Syringe Filter (0.45 μm) and the filtrate was made to 5 ml with n-hexane and stored at 4 °C**.**

GC-MS Analysis: The essential oil in the leaf litter was analysed by Gas chromatography - Mass spectrometry (GC-MS) (17) with a modification of oven temperature-rising procedure in GC: The oven temperature was held at 60 °C and programmed to rise to 100 °C at a rate of 10 °C min^{-1}, followed by an increase to 180 °C at a rate of 5 °C min^{-1}, and followed by an increase to 300 °C at a rate of 20 °C min^{-1}. Identification of compounds was based on comparisons of the mass spectra with published data (17,38,40,43) and records in the NIST (National Institute of Standards and Technology) 08 Databases.

Statistical Analyses

The differences in the morphological indicators between leaf litter treated and control plants were examined by T-test using the SPSS 16.0 statistical analysis software (SPSS Inc., USA). ANOVA was used to analyse the main effects of leaf litter, time, and their interactions effects. The quantified models of the flowering dynamics of two species were built by running non-linear regression program of SPSS.

RESULTS AND DISCUSSION

I. Height, root collar and biomass

Balsamine: The camphor leaf litter during the initial stages of decomposition (till 60 days) significantly inhibited the height and root collar growth of Balsamine. At the 25th, 39th and 54th days, the reduction was 9.5 %, 42.7 % and 62.6 % in height, and reduction of 20.5%, 58.9% and 71.3% in root collar, respectively, than control. There were significant interaction effects of leaf litter × decomposition time on height and root collar growth (Table 1).

Morning glory: Similar results were observed but the leaf litter inhibition from the decomposed leaf litter was over longer period (16 to 97 days) of decomposition. The decrease in height, root collar, shoot biomass and root biomass were 57.2 %, 34.4 %, 80.9 % and 70.1 %, respectively, than control (Table 1).

II. Leaf development

Balsamine: It had less and smaller leaves in leaf litter treatment, and the differences with control were time dependent, and increased with decomposition time (52 days). Interestingly, the leaf numbers of plants in leaf litter treated soil was only 16.1 % than control at the 52^{nd} day. The cotyledon and the first euphylla area were reduced by 33.5 % and 85.1 %, respectively, with leaf litter incorporation (Table 2).

Morning glory: The camphor leaf litter inhibited the leaf development. Its cotyledon petiole length, cotyledon area, first euphylla area and leaf number were reduced by 37.7 %, 48.7 %, 83.6 % and 38.6 %, respectively, at 17, 17, 20, 46 days after sowing (Table 2).

Table 1. Effects of Camphor leaf litter on height, root collar and biomass accumulation of Balsamine and Morning glory

Camphor litter	Height (cm)			Root collar (mm)			Biomass(g)	
	Balsamine							
	25d	39d	54d	25d	39d	54d	-	-
Control	4.00	7.75	23.02	2.34	4.79	11.79	-	-
Added in pots	3.62*	4.44*	8.62*	1.86*	1.97*	3.38*	-	-
% I/S over control	-9.5	-42.7	-62.6	-20.5	-58.9	-71.3	-	-
RM-ANOVA	$p_{CL}<0.001$	$p_t=0.001$	$p_{CL\times t}<0.001$	$p_{CL}<0.001$	$p_t<0.001$	$p_{CL\times t}<0.001$	-	-
	Morning glory							
	16d	46d	97d	16d	46d	97d	S 97d	R 97d
Control	3.11	29.50	176.42	3.07	4.50	4.82	15.45	3.48
Added in pots	3.23NS	9.00*	75.58*	2.66*	3.32*	3.16*	2.95*	1.04*
% I/S over control	+3.9	-69.5	-57.2	-13.4	-26.2	-34.4	-80.9	-70.1
RM-ANOVA	$p_{CL}=0.009$	$p_t=0.001$	$p_{CL\times t}=0.018$	$p_{CL}<0.001$	$p_t<0.001$	$p_{CL\times t}=0.007$	-	-

Measuring time was calculated from the sowing day (camphor litter added to pots), n=3; % I/S: % Inhibition (-)/Stimulation (+) over control; S: Shoot, R: Root, p_{CL}: Camphor litter effect, p_t: Time effect, $p_{CL\times t}$:: Interaction effect, NS: Non-Significant, * : Significant difference, - : Not determined.

Table 2. Effects of applied camphor litter on leaf development of balsamine and morning glory.

Camphor litter	Leaf number			Leaf area (cm^2)		Petiole length (mm)
	Balsamine					
	32d	39d	52d	Cotyledon 18d	1st euphylla 24d	-
Control	11.67	19.56	98.33	2.45	4.50	-
Added in pots	7.50*	9.44*	15.83*	1.63*	0.67*	-
% I/S over control	-35.7	-51.7	-83.9	-33.5	-85.1	-
RM-ANOVA	$p_{CL}<0.001$	$p_t<0.001$	$p_{CL\times t}<0.001$	-	-	-
	Morning glory					
	46d			Cotyledon 17d	1st euphylla 20d	17d
Control	11.67			13.75	16.80	28.42
Added in pots	7.17*			7.05*	2.76*	17.71*
% I/S over control	-38.6			-48.7	-83.6	-37.7

Measuring time was calculated from the sowing day (camphor litter added to pots), n=3; % I/S: % Inhibition (-)/ Stimulation (+) over control; p_{CL}: Camphor litter effect, p_t: Time effect, $p_{CL\times t}$:: Interaction effect, * : Significant difference, - : Not determined.

The decomposing camphor leaf litter greatly influences the primary growth of herbaceous plants (32,41), which agrees with similar studies using litter powder. The mechanism underlying the detrimental effects is probably linked to a multistep and multilevel action pathway of allelochemicals, from limitation of soil available nitrogen (8,48), to lipid peroxidation caused by reactive oxygen species (ROS) (6,28,45), and to depression of photosynthetic capacity (2,16,27), as revealed thoroughly previously. Despite the complexity of the mechanism, it could be speculated that the receiver plants tend to be influenced by the induced ROS at early growth stages (relatively lower stress resistance and nutrition demand), and instead, by soil nitrogen limitation as they grow (higher stress resistance and nutrition demand). Imaginably, the subsequent reproductive growth is likely to be affected.

III. Flowering dynamics

Balsamine: A weaker flowering vigour was observed after exposure to the leaf litter. There was a delay of 7 days and decrease of 18.5 flowers per plant in the AF_{max} and a fall of 1.7 flowers per plant per day in the FV_{max} were estimated from the fitted equations in treatments. Besides, at 3- months after sowing, all control plants bloomed, while only one-sixth of leaf litter treated plants bloomed (Fig. 2 Fig. 3 and Table 3). The decreased flowers number, reduced the fruits and seeds yield, regardless of pollination and soil nutrients supply, while the delay in flowering affected the plant regeneration and succession.

Figure 2. The flowering of Balsamine and Morning glory.

Morning Glory: The morning glory was less sensitive than balsamine, its flowering was not greatly affected, but still, the AF_{max} was reduced by 3.3 flowers per plant and the FV_{max} was only half of control (Fig. 2 Fig. 3 and Table 3). Moreover, interestingly, the treated morning glory plants had smaller flowers at relatively higher node positions (11^{th}~14^{th} nodes) (Fig. 4). Possibly, morning glory tried to keep normal reproductive growth under the allelopathic stress of nutrients deficiency [nitrogen (8) and potassium, calcium, ferrum and manganese (12)].

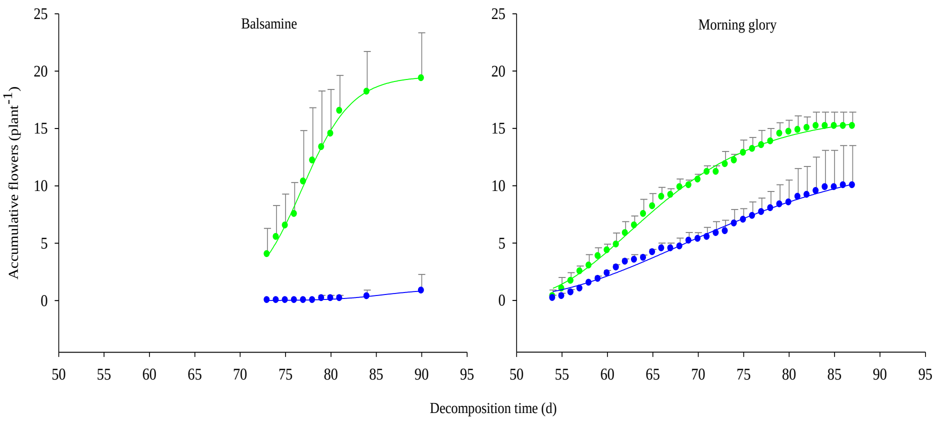

Figure 3. The flowering dynamics of Balsamine and Morning glory.
Scatters were plotted with positive error bars. Green dots: Control, blue dots: +Camphor leaf litter

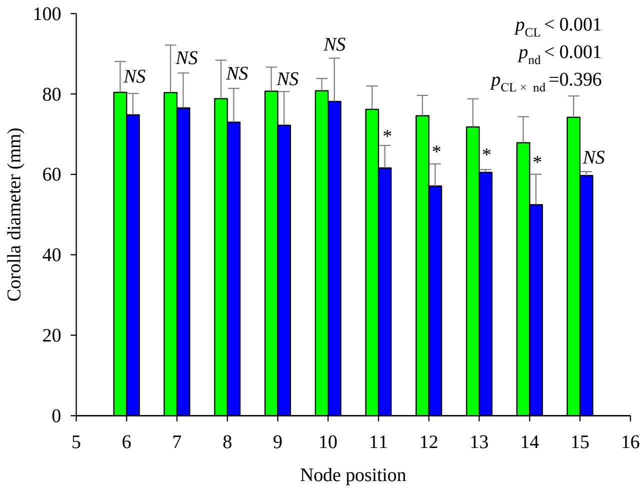

Figure 4. Effects of leaf litter on corolla size of Morning glory.
Columns were graphed with positive error bars. Green column: Control, blue column: Camphor litter added in pots. NS: Non-Significant, *: Significant difference, p values [$p_{CL:}$ Camphor litter effect, p_{nd} : Node position effect and $p_{CL\times nd}$, : Interaction effect, respectively] were given by Univariate two-factor ANOVA.

The leaf litter of *Cinnamomum japonicum* reduced the flowering velocity and number of flowers in balsamine, but it did not influence the flowering duration (2). These results might link to imbalance of hormones. It was found the production of indole-3-acetic acid was decreased, while abscisic acid was increased in wheat, in response to sunflower (*Helianthus annuus* L.) extracts (23). The balsamine showed markedly lower ratio of soluble sugar and soluble protein in leaves due to camphor leaf litter (29). The spinach (*Spinacea oleracea* L.) bolted and bloomed earlier after treated with leaf litter of *Eucalyptus globulus* Labill. subsp. *maidenii* (F. Mueller) Kirkpatrick, and there was rise in soluble sugar/soluble protein ratio (6). The application of walnut (*Juglans regia* L.) and pine (*Pinus* sp.) leaf extracts reduced the vegetative growth and grain yield of wheat, but the development of wheat was not influenced (1).

Table 3. Effects of applied camphor litter on flowering parameters of balsamine and morning glory.

Camphor litter	D_{first} (d)	D_{last} (d)	*FD* (days)	AF_{max} ($plant^{-1}$)	FV_{max} ($plant^{-1} \cdot d^{-1}$)	*FP* (%)	Flowering model
	Balsamine						
Control	66.68	87.01	20.33	19.56	1.78	100	$19.558/[1+5.859e^{-0.365(x-72)}]$ n=66, r^2=0.258, F=10.938, p <0.001
Added in pots	-79	-	-	1.02	0.09	16.67	$1.021/[1+15.325e^{-0.350(x-78)}]$ n=66, r^2=0.258, F=4.372, p=0.017
	Morning glory						
Control	51.4	82.36	30.96	16.23	0.71	100	$16.226[1-e^{-0.119x}]^{1712.825}$ n=102, r^2=0.968, F=1516.373, p<0.001
Added in pots	54.11	88.52	34.41	12.93	0.35	100	$12.930[1-e^{-0.074x}]^{150.991}$ n=102, r^2=0.835, F=251.161, p <0.001

D_{first} : The day the first flower appeared in group, calculated from the sowing day (camphor litter added to pots), D_{last} : The day last flower bloomed in group, *FD*: Flowering duration, D_{last} - D_{first}, AF_{max} : Maximum accumulated flower number per plant, FV_{max}: Maximum flowering velocity, *FP* : Flowering percentage at last observation. AF_{max}: Values were obtained from the fitted Logistic equations. The other parameters, except *FP*, were obtained from the corresponding first-order derivation. Data in the parentheses were based on actual observation. - : Not determined.

IV. Seed quality and germination

Balsamine: The leaf litter neither greatly influenced the 1000-grain weights nor the germination (%) of harvested seeds. Notably however, more deformed-cotyledon sprouts emerged from leaf litter treatment than from control, which did not happen to morning glory (Table 4).

Morning Glory: The leaf litter slightly influenced the 1000-grain weights and the germination (%). The seeds harvested from the 7^{th} to 9^{th} nodes had higher germination than from 4^{th} to 6^{th} nodes, regardless of treatment (Table 4).

These results were perhaps due to a kind of adaption of plants to adverse environment, and there might be reproductive improvement during the flowering/yield period. The single fruit weight and the 1000-grain weight of *Capsicum annuum* were hardly altered by camphor leaf litter at the same dose, despite 31 % decrease in fruits yield per plant (5). The seed weight per capsule and 100-grain weight of Balsamine were slightly affected by the leaf litter of *C. japonicum* (2).

Table 4. Effects of Camphor leaf litter on harvested seed weight and germination (%) of Balsamine and Morning glory.

Camphor litter	1000-grain weight (g)		Germination (%)	
	Balsamine			
Control	5.6		74.00 (19.02)	
Added in pots	4.87NS		74.00NS (68.18*)	
% I/S over control	-13.0		0.0 (+258.5)	
	Morning glory			
	4th-6th node	7th-9th node	4th-6th node	7th-9th node
Control	43.57	46.67	72.22	96.67
Added in pots	46.67NS	43.73NS	76.67NS	90.48NS
% I/S over control	+7.1	-6.3	+6.2	-6.4

n=3~5; % I/S: % Inhibition (-)/Stimulation (+) over control; NS: Non-Significant, * : Significant difference; Data in the parentheses show percentages of abnormal sprouts featuring deformed cotyledons.

V. Harvested seeds growth performance

Balsamine: The growth vigour of progeny of leaf litter treated Balsamine was weaker than control, in terms of height in the first 37 days and root collar in the first 63 days. It seemed that some physiological changes occurred in the leaf litter treated seeds of Balsamine, in combination with higher percentage of deformed-cotyledon sprouts. The total soluble solid and vitamin C of strawberry (*Fragaria* × *ananassa* L.) was significantly reduced after exposure to juglone and walnut leaf extracts (12). There was only 15.9 % decrease in height and 16.6 % decrease in root collar than control. Later, the treated Balsamine progeny recovered gradually to the control level (Fig. 5 and Table 5).

Table 5. Effects of Camphor leaf litter on growth traits of the progeny of Balsamine and Morning glory.

Camphor litter	Height (cm)				Root collar (mm)			
	Balsamine							
	30d	37d	49d	63d	30d	37d	49d	63d
Control	7.5	16.39	26.41	46.71	4.20	6.75	9.74	13.29
Added in Pots	5.00*	12.71*	24.73NS	45.96NS	3.26*	5.41*	8.49*	11.78*
% I/S over control	-33.3	-22.5	-6.4	-1.6	-22.4	-19.9	-12.8	-11.4
RM-ANOVA	p_{CL}=0.092, p_t<0.001, $p_{CL\times t}$=0.189				p_{CL}=0.003, p_t<0.001, $p_{CL\times t}$=0.616			
	Morning glory							
	27 d				37 d			
	4th-6th node		7th-9th node		4th-6th node		7th-9th node	
Control	6.59		6.99		3.47		3.41	
Added in Pots	6.88NS		7.13NS		3.35NS		3.55NS	
% I/S over control	+4.4		+2.0		-3.5		+4.1	

Measuring time was calculated from the sowing day (camphor leaf litter added to pots), n=3; % I/S: % Inhibition (-) /Stimulation (+) over control; p_{CL}: Camphor litter effect, p_t: Time effect, $p_{CL\times t}$:: Interaction effect, NS : Non-Significant, * : Significant difference.

Morning Glory: Comparatively, both early growth traits of progeny of leaf litter treated Morning glory were statistically similar to control, followed by synchronous flowering phenology (Fig. 5 and Table 5). These results differed from that observed with the parent plants at respective growth stages, as stated previously and similar regularity was observed in *Capsicum annuum* as affected by decomposing leaf litter of camphor tree (5).

Figure 5. The growth performance of Balsamine and Morning glory grown from harvested seeds.

Balsamine: Plants grown from harvested seeds of camphor leaf litter treated plants (left), and plants grown from harvested seeds of control plants (right). **Morning glory:** From left to right, plants grown from the seeds harvested at the 4th to 6th nodes of camphor leaf litter treated plants, at the 4th to 6th nodes of control plants, at the 7th to 9th nodes of camphor leaf litter treated plants, and at the 7th to 9th nodes of control plants, respectively.

VI. Potential allelochemicals

In this study, the vegetative growth and flowering traits of two test ornamental plants were affected by the decomposing leaf litter of camphor tree, but what component in leaf litter was responsible had to be determined. From the results of GC-MS and supplementary trial, we suspect that five terpenoid compounds [Camphor (15.18 %), 1,8-Cineole (13.6 %), α-Terpinenol (7.96 %), Sabinene (6.17 %) and β-Caryophyllene (4.95 %)] in the essential oil were perhaps responsible for these results. Because they (i). constituted a higher content in the essential oil of leaf litter and (ii). their content decreased sharply or even disappeared completely after decomposition for 4.5 months. It is reported that the decomposed leaf litter did not exert any effects on the growth of hot pepper (5). Further we found that three compounds (Camphor, 1,8-Cineole and β-Caryophyllene) were relatively stable in leaf litter for many years. In 2013-leaf litter sample, 31 terpenoids, including 2 chain, 3 oxygenated chain, 13 cyclic and 13 oxygenated cyclic molecules were identified, representing 67.1 % of the essential oil**.** The essential oil was dominated by Camphor (15.5 %), 1,8-Cineole (7.09 %) and β-Caryophyllene (4.61 %, accompanied by 2.97 % of α configuration) (Table 6). In fact, these three compounds (Camphor, 1,8-Cineole, β-Caryophyllene) are suppressive to herbs and microbes in indoor bioassays (9,24,32,35,42) and are allelopathic (4,39).

Table 6. The terpenoid compounds identified in camphor leaf litter using GC-MS.

Compounds	**Molecular formula**	**Relative content (Absolute peak area)**
Chain terpenes		
Myrcene	$C_{10}H_{16}$	0.26(2253914)
Neophytadiene	$C_{20}H_{38}$	0.35(3090328)
Subtotal		**0.61(5344242)**
Oxygenated chain terpenes		
Linalool	$C_{10}H_{18}O$	14.18(124819301)
Terpendiol I	$C_{10}H_{18}O_2$	0.22(1936957)
Phytol	$C_{20}H_{40}O$	1.38(12176419)
Subtotal		**15.78(138932678)**
Cyclic terpenes		
α-Pinene	$C_{10}H_{16}$	0.17(1479131)
Sabinene	$C_{10}H_{16}$	0.81(7087504)
β-Pinene	$C_{10}H_{16}$	0.23(1980979)
α-Terpinolene	$C_{10}H_{16}$	0.20(1787284)
Limonene	$C_{10}H_{16}$	0.46(4041198)
β-Elemene	$C_{15}H_{24}$	0.21(1857718)
β-Caryophyllene	$C_{15}H_{24}$	4.61(40561651)
Germacrene D	$C_{15}H_{24}$	1.69(14905768)
β-Selinene	$C_{15}H_{24}$	0.29(2509240)
Bicyclogermacrene	$C_{15}H_{24}$	2.69(23710121)
γ-Gurjunene	$C_{15}H_{24}$	0.33(2931849)
α-Caryophyllene	$C_{15}H_{24}$	2.97(26122513)
Germacrene B	$C_{15}H_{24}$	0.64(5590764)
Subtotal		**15.28(134565719)**
Oxygenated cyclic terpenes		
1,8-Cineole	$C_{10}H_{18}O$	7.09(62440466)
trans-4-Thujanol	$C_{10}H_{18}O$	0.41(3609784)
cis-4-Thujanol	$C_{10}H_{18}O$	0.19(1655218)
Camphor	$C_{10}H_{16}O$	15.57(137074960)
L(-)-Borneol	$C_{10}H_{18}O$	3.14(27601644)
Terpinen-4-ol	$C_{10}H_{18}O$	0.44(3873915)
α-Terpinenol	$C_{10}H_{18}O$	3.92(34548278)
L-Bornyl acetate	$C_{12}H_{20}O_2$	1.09(9570331)
Caryophyllene oxide	$C_{15}H_{24}O$	0.61(5388264)
Humulene oxide	$C_{15}H_{24}O$	0.27(2385979)
Spathulenol	$C_{15}H_{24}O$	0.76(6726525)
γ-Sitosterol	$C_{29}H_{50}O$	1.70(14976203)
4,5-epoxy-1-isopropyl-4-methyl-1- cyclohexene	*	0.25(2157066)
Subtotal		**35.44(312008634)**
Total		**67.11(590851273)**

* : The molecular formula was not ascertained.

Inderjit and Nilsen (19) proposed that a control using well-washed materials or materials from some putatively non-allelopathic species is needed in experiments exploring allelopathy from plant residues degradation. Given this, several experiments were done in which (i). the steam-distilled leaf litter (2,29), (ii). leaf litter buried in soil for 4~4.5 months and (iii). high-density plastic film (5,8) were used. These substitutes did not exert remarkable affects, implying that changes in the soil physico-chemical properties (aeration, water movement, carbon/nitrogen ratio, nitrogen immobilization caused by soil microbes, etc.) could not explain the effects of added leaf litter. Therefore, allelopathy from the decomposing litter is critical factor. Moreover, we have found in very few cases that decomposing litter of donor plants slightly inhibited the growth of receiver plants (21). This might be attributed to the background of soil used, because the allelopathic activity may vary with soil type (14), soil microflora (18) and soil fertility (37,45). This provides guidelines for the integrated management of allelopathic leaf litter and soil environment in specific systems. Considering that allelochemicals are probably transformed by soil biochemical processes (10,22,25), further detailed research is needed to discover the fates of these chemicals present in leaf litter and in the soil, how they interfere directly with root meristems of plants, how they change the soil microbial communities associated with soil nutrients supply etc.

CONCLUSIONS

The decomposing leaf litter of camphor tree at the application rate of 0.56% (w/w) was harmful not only to the vegetative growth but also to the reproductive phases of test plants: Balsamine and Morning glory. It is hypothesized that 3- terpenoid compounds (Camphor, 1,8-Cineole and β-Caryophyllene) present during the initial stage of leaf litter decomposition, were responsible for the allelopathic effects. In landscape or agroforestry systems, these plants grow with *Cinnamomum* species. The allelopathic camphor tree leaf litter added into the soil (mass fraction ≥ 0.56 %) slightly inhibited the harvested seeds quality, germination and seedlings growth and also decreased their ornamental or economic values. Thus, it is advised to remove Camphor leaf litter after its higher leaf fall and to prepare its compost, before adding to soil.

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