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SHORT COMMUNICATION

Effects of tree species on seed germination and seedlings growth of Chinese medicinal herb *Gentiana rigescens*

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ABSTRACT

Allelopathic effects of 4-tree species [*Camellia sinensis* (L.) O. Ktze, *Eucalyptus robusta* Smith, *Chaenomeles sinensis* (Thouin) Koehne, *Alnus nepalensis* D. Don] were studied on Chinese medicinal plant, *Gentiana rigescens* Franch. ex Hemsl.. The leaf or root aqueous extracts of all tree species reduced the germination, plumule and radicle growth of *G. rigescens,* but the effects were variable. The highest seed germination rate was with leaf extracts (10.0%) of *Camellia sinensis.* Contrarily the seeds did not germinate in leaf extracts of *Eucalyptus robusta* and *Alnus nepalensis*. The longest plumule length (3.37 mm) was in leaf extracts of *Camellia sinensis*. Hence *G. rigescens* may be grown as intercrop in tea (*Camellia sinensis*) plantation.

Key words: Agroforestry, allelopathy, *Alnus nepalensis*, *Camellia sinensis*, *Chaenomeles sinensis*, economic forest, *Eucalyptus robusta*, *Gentiana rigescens*, medicinal plant

INTRODUCTION

Medicinal plants play major role in healthcare of people and in trade of commodities (15). Many medicinal plants growing under forest cover are shade tolerant, but forests are over-harvested, thereby putting the medicinal plants at risk (11, 15). The mono-cultivation of medicinal plants is not good option, due to very small land holdings of farmers and long gestation period (12).

Agroforestry systems improves the food security and reduce the environmental degradation, as well as provide various products for households and medicinal purposes (10). Therefore, medicinal plants cultivation in agroforestry is an attractive strategy to promote their cultivation and conservation, due to much lower production cost than in nursery and they provide secondary income to farmers (11). For example, medicinal plants could be successfully grown as intercrops in arecanut (*Areca catechu* L.) plantation with

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higher productivity and net income per unit area (21). The medicinal plants (*Ocimum basilicum* L. and *Tagetes minuta* L.) could be grown successfully with *Morus* hedgerows (18). In North America, American ginseng (*Panax quinquefolium* L.), is grown as an understorey herb in sugar maple (*Acer saccharum* Marshall) or red oak (*Quercus rubrum* L.) forests, instead of growing under artificial shade (9).

Yunnan Province, China, is rich in cultural and biological diversity (7). Agroforestry is important for sustainable upland management and for increasing the productivity under population pressure (6). *Gentiana rigescens* Franch. ex Hemsl. is a medicinal plant endemic to southwest China and the wild *G. rigescens* grows in mountains forest in Yunnan (20). As a traditional Chinese medicine, *G. rigescens* root is commonly used to treat inflammation, hepatitis, rheumatism and cholecystitis (4). The dammarane triterpenoids from the roots of *G. rigescens* have antifungal activity against plant pathogens (24). This species is threatened by over-exploitation due to commercial harvest of forest for large-scale trade (8). Therefore, farmers began to grow *G. rigescens* in monoculture since 1998 (19). Recently, the practice of growing *G. rigescens* has re-appeared with some tree species.

In this study, the effects of *Camellia sinensis* (L.) O. Ktze, *Eucalyptus robusta* Smith, *Chaenomeles sinensis* (Thouin) Koehne, and *Alnus nepalensis* D. Don were studied on germination and seedlings growth of *G. rigescens* in agroforestry systems. The tea plant (*Camellia sinensis*) is cultivated in China since > 2000 years and is important beverage crop worldwide. Yunnan 'Pu'er' Tea, is exported to many countries, hence, plays an important role in economy (3). *Eucalyptus species*, like *E. robusta* was introduced and planted in large areas in China due to its fast growth, hence, brought considerable benefits to tree farming industry (5,26). The *Chaenomeles sinensis* tree is mainly distributed in China, Korea and Japan. Its fruits (rich in phenolics) are used to treat throat diseases (17). *A. nepalensis* is a multipurpose fast growing tree, planted to control soil erosion on hillsides and for land recovery in shifting cultivation (2).

MATERIALS AND METHODS

The fresh, mature leaves and roots of 4-tree species [*Camellia sinensis*, *E. robusta*, *Chaenomeles sinensis* and *A. nepalensis*] and seeds of *G. rigescens* were collected from their natural habitat in Lincang, Yunnan in February 2011. The seeds of *G. rigescens* are very small (1.5-2.5 mm dia. and 14-29 mg 1000- kernel weight) (25).

The leaves or roots were dried and 2.0 g powder of each sample was added to 100 ml-distilled water and left for 48 h at room temperature (12). The extracts were filtered through 3-layers of filter paper and stored in dark, until used. Fifty seeds were placed in sterilized petri dishes lined with two layers of filter paper. The petri dishes were saturated with respective extract using 5.0 ml extract of each plant and distilled water was used as control. The treatments were replicated 4-times. Moisture in the petri dishes was maintained by adding 1.0 ml extract or distilled water as required. Seed germination was counted daily, while plumule and radicle growth were recorded at 20 and 26 day. The data were statistically analyzed using one-way ANOVA with LSD post hoc analysis.

RESULTS

Seed germination

The seeds germination of *G. rigescens* in control (distilled water) occurred between 11-26 days after sowing (Fig. 1). The final seeds germination was 60%. The leaf aqueous extracts of tree species were more inhibitory to germination of *G. rigescens* than root aqueous extracts. The inhibition of each tree specie's leaf aqueous extract was significant higher than the root aqueous extracts. The extracts of all tree species were inhibitory to seeds germination of *G. rigescens* [Except stimuation (22%) at 11 DAS (Days after sowing) with *Camellia sinensis* root extract]. Leaf extracts of *E. robusta* and *A. nepalensis* proved most deleterious and completed inhibited the seeds germination of *G. rigescens*.

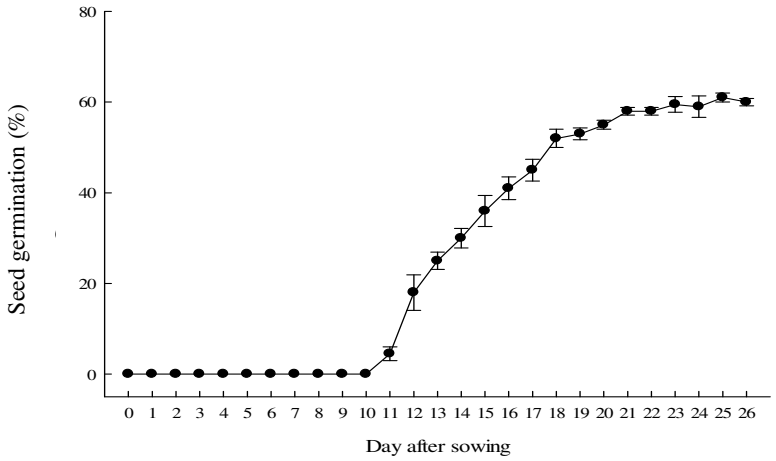

Figure 1 Germination rate of *Gentiana rigescens* in distilled water (Control).

Seedling growth

The root or leaf extracts of all tree species drastically reduced the radicle length of *G. rigescens* seedling but the degree of reduction was variable (Table 1). The lowest inhibition with leaf extract on plumule length was found in *C. sinensis* treatment (30.5% at 20 DAS and 39.1% at 26 DAS). Whereas, the lowest inhibition with root extract on plumule length was found in *A. nepalensis* treatment (32.8% at 20 DAS and 21.9% at 26 DAS). The radicles were less than 1.0 mm in length with extracts of all tree species. The root or leaf extracts of all tree spp. drastically reduced the root length (Table 2). The roots were less than 1.0 mm in length with extracts of all tree spp.

Table 1. The inhibitory effects (%) of aqueous extracts of trees on plumule length (mm) of *Gentiana rigescens* seedling

Day	*Camellia sinensis*		*Eucalyptus robusta*		*Chaenomeles sinensis*		*Alnus nepalensis*	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
20	30.5	>71.8*	100.0	>71.8*	>71.8*	>71.8*	100.0	32.8
26	39.1	46.8	100.0	37.3	81.9	49.6	100.0	21.9

*Value is less than 100.

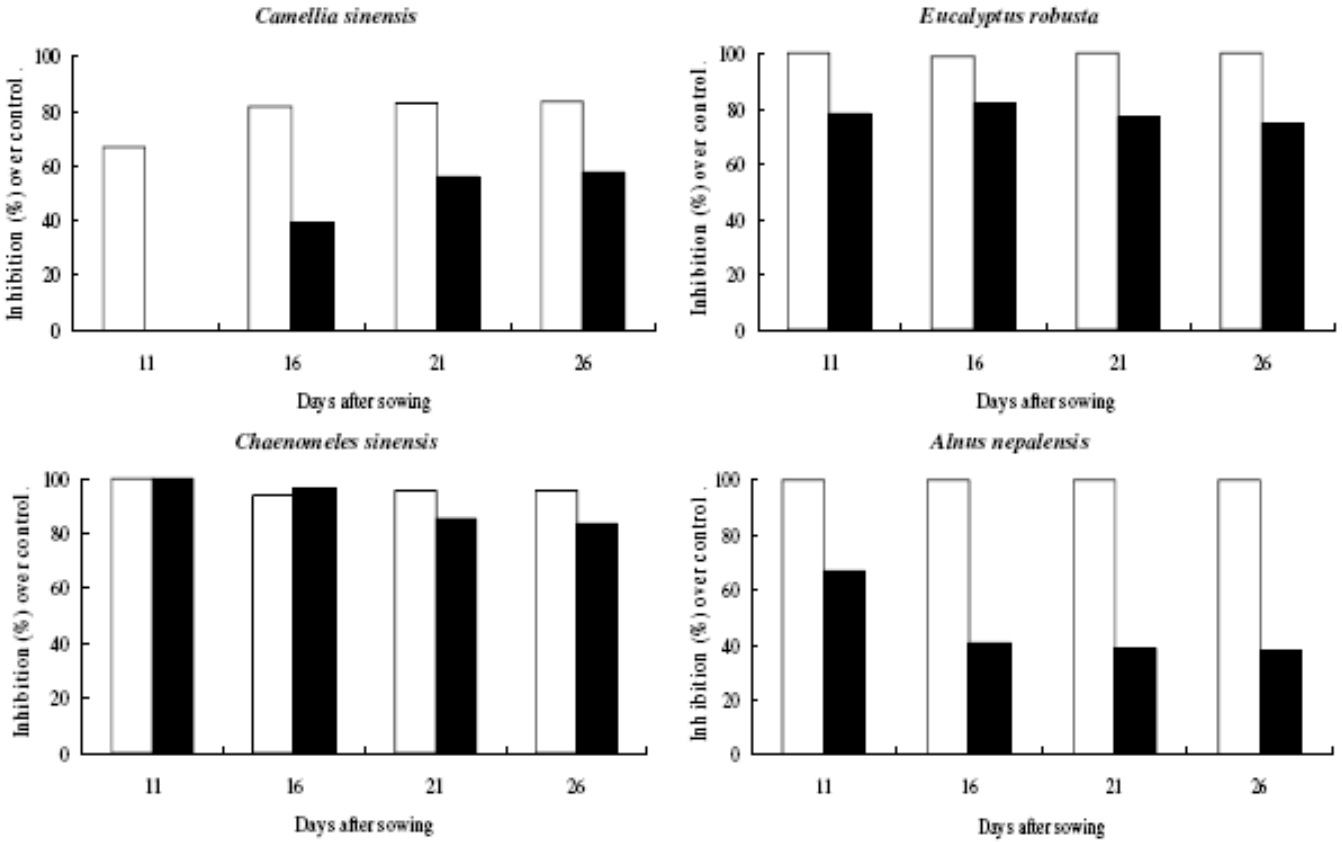

Figure 2 Inhibitory effects of leaf and root extract of (a) *Camellia sinensis*, (b) *Eucalyptus robusta*, (c) *Chaenomeles sinensis* and (d) *Alnus nepalensis* on seeds germination of *Gentiana rigescens*. □ Leaf extract, ■ Root extract.

Table 2. The inhibitory effects (%) of aqueous extracts of different trees on radicle length (mm) of *Gentiana rigescens* seedling

Day	*Camellia sinensis*		*Eucalyptus robusta*		*Chaenomeles sinensis*		*Alnus nepalensis*	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
20	>71.8*	>71.8*	100.0	>71.8*	>71.8*	>71.8*	100.0	>71.8*
26	>71.8*	>71.8*	100.0	>71.8*	>71.8*	>71.8*	100.0	>71.8*

*Value is less than 100.

DISCUSSION

The cultivation of medicinal plants requires in-depth knowledge of many variables including allelopathy, to improve the quality of medicinal plants (12). Leaf extract are more allelopathic to germination, initial germination time, speed of germination than root extract (22,23). The seed germination of *G. rigescens* was found sensitive (germination and seedling growth) to 2% leaf or root aqueous extracts of all four tree species. The aqueous extract of *Eucalyptus* plants inhibits the germination and seedling growth of other species (5,26). High concentration of leaf extract of *A. nepalensis* has negative impacts on *Pinus yunnanensis* seed germination (1).

The leaf extracts of *Camellia sinensis* had weaker effects compared with flowers and fruits extracts on germination of *Amaranthus retroflexus*. and *Setaria glauca* (16). However, the leaves and leaf buds of *C. sinensis* are used to produce Chinese tea; its allelopathic effects may be lower in the field (14). Survey study showed that the active compound contents in the root of *G. rigescens* grown in tea plantation were not significantly lower than monocuture (P>0.05) (19)

The shoot aqueous extract of *Tithonia diversifolia* have different effects (inhibitory and stimulatory) on seedling growth of *Zea mays*, depending on plants growth stage (13). Transplanting of seedling is the best approach to establish the species with low germination (26). The gibberellic acid increased the germination to 95% and reduces the *G. rigescens* seed germination time (25), hence, the plantations of *C. sinensis* can be established by transplanting the *G. rigescens* seedlings treated with gibberellic acid.

This study was done to investigate the allelopathic behaviour of *C. sinensis*, *E. robusta*, *Chaenomeles sinensis* and *A. nepalensis* trees on germination and growth of *G. rigescens*, to develop the agroforestry practices. The 2% leaf or root aqueous extracts of all 4-tree species reduced the germination, plumule and radicle growth of *G. rigescens*. Our results suggested that *G. rigescens* can be grown as Agroforestry component with *Camellia sinensis*. However, further field studies are required as understory of tree species in natural conditions to fully understand the allelopathic interactions of these tree species. For marginal farmers, monoculture of medicinal plants is not good option, hence, medicinal plant based agroforestry are being developed.

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