Songklanakarin J. Sci. Technol.
41 (2), 465-470, Mar. - Apr. 2019

http://www.sjst.psu.ac.th

Original Article

Effects of invasive *Ageratina adenophora* on mycelial growth of some important soil fungi

Sujan Balami*, Lal B Thapa, and Sanjay Kumar Jha

Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, 44600 Nepal

Received: 11 November 2016; Revised: 23 October 2017; Accepted: 12 December 2017

Abstract

Ageratina adenophora, an invasive alien plant of neo-tropical origin, is known to have adverse impacts on native species, soil nutrients and soil microbial activities. Impact of *A. adenophora* on ecologically important soil fungi is crucial to know how it modifies soil microbial community during invasion. We studied mycelial growth of selected ecologically important soil fungi (*Alternaria alternata*, *Trichoderma harzianum*, *Aspergillus niger*, *Chaetomium funicola* and *Fusarium oxysporum*) by using extracts obtained from *A. adenophora* leaves, litter and root. All types of extracts showed inhibitory effect on fungal growth. The degree of inhibition varied with fungal species, extract type and concentration. It is proposed that the inhibitory activities of *A. adenophora* to the soil fungi could bring changes in soil fungal diversity and their composition. It could be a reason that affects soil characteristics, native plant species and ecosystem functioning by *A. adenophora*.

Keywords: Crofton weed, extracts, poison food technique, antifungal activity

1. Introduction

Ageratina adenophora (Sprengel) R. King & H. Robinson, the Crofton weed, is a perennial sub-shrub natively found in Mexico (King & Robinson, 1970). It is invasive to more than 30 countries including China, India, Indonesia, Vietnam, Nepal and Maynmar (Qiang, 1998; Tiwari *et al.*, 2005). Invasion of *A. adenophora* has become a serious threat to native ecosystem dynamics and biodiversity in its non-native range (Bajpai & Indrerjit, 2013; Thapa & Maharjan, 2014, 2015). In Nepal it has been invading road side, fallow lands, forest margins or disturbed areas (Thapa *et al.*, 2016a, 2017; Tiwari *et al.*, 2005). *A. adenophora* is responsible to alter below ground microbial communities which are considered as one of the important mechanisms behind its invasion success (Beest *et al.*, 2009; Inderjit & Cahill, 2015). The mechanism includes changes in soil microbial community composition, belowground mutualism, mycorrhizal association and accumulation of plant pathogens in invaded soil (Cantor *et al.*, 2011; He *et al.*, 2009; Lorenzo *et al.*, 2010).

*Corresponding author
Email address: balamisujan@gmail.com

Previous works highlighted that *A. adenophora* is associated with high nutrient accumulation in invaded soil (Niu *et al.*, 2007; Sun *et al.*, 2013). Moreover, soil VAM (vesicular-arbuscular mycorrhizal fungi) can be significantly increased by *A. adenophora* invasion (Niu *et al.*, 2007). On the other hand, *A. adenophora* also decreases the biomass of soil microbes; gram negative bacteria and some mycorrhizal or non-micorrhizal fungi (Sun *et al.*, 2013). Various secondary metabolites such as quinic acid derivatives and sesquiterpenes that are found in *A. adenophora* (Kundu *et al.*, 2013; Zhang *et al.*, 20 13) possesses antibiosis properties that may influence fungal or bacterial growth and development (Ha *et al.*, 2003; Zhang *et al.*, 2013).

Our study aimed to know the effect of *A. adenophora* on selected beneficial or pathogenic soil fungi. We hypothesized that the effect of extracts of different parts of *A. adenohora* to soil fungi is species specific and then it takes benefit from its antifungal activities against either pathogenic or beneficial fungi in native soil. We selected ecologically important five soil fungi, *Alternaria alternata*, *Aspergillus niger*, *Trichoderma harzianum*, *Fusarium oxysporum* and *Chaetomium funicola*. Among them *A. alternata* and *F. oxysporum* are common plant pathogens that cause damping off of seedlings in many plants (Agrios, 2005). *Trichoderma*

harzianum involves in decomposing large number of recalcitrant substances in forest floor, *A. niger* is the common decomposer (Thom & Ra-per, 1945) and *C. funicola* is known to have antibacterial and antifungal properties (Istifadah *et al.*, 2006; Pietro *et al.*, 19 92).

2. Materials and Methods

2.1 Test fungi

Fungal species selected for the study were *Alternaria alternata, Aspergillus niger, Trichoderma harzianum, Fusarium oxysporum* and *Chaetomium funicola* on the basis of their role as plant pathogens or decomposers. The species were isolated from the *A. adenophora* invaded and non-invaded soils by serial dilution method (Aneja, 2003) and their pure cultures were obtained.

2.2 Extract preparation

Ageratina adenophora root, fresh leaves and litter were collected from invaded sites nearby the Central Department of Botany Garden, Tribhuvan University, Kathmandu, Nepal. From the collected parts extracts were prepared. Fifteen gram of each part was soaked in sterile distilled water for 48 hrs and then filtered through 5 layered muslin cloths. The extracts obtained was then centrifuged to remove debris and then filtered through Whatman No. 1 filter paper. A total three concentrations of the each type of extract were made, the stock solution (100%) which was diluted to 50% and 25%. The solutions were stored at 4 ^{o}C until use.

2.3 Fungal treatment

Fungal treatments were done following the Poison food techniques (Grover and Moore, 1962). The extracts of *A. adenophora* were amended on the Potato Dextrose Agar (PDA) plates thereby each PDA plate contained extract and PDA in ratio 1:4 (10 ml extract and 40 ml PDA). Test fungi were cultured on Petri-plates containing *A. adenophora* extracts (0, 25, 50 and 100%). Control set was maintained by amending PDA media (40 ml) with 10 ml distilled water.

During the treatment 5 mm^2 sized actively growing mycelial plug of each fungus was aseptically inoculated on the PDA media containing *A. adenophora* extract. Each treatment had five replicates. The plates were incubated at 25°C in the incubator. After a week, maximum and minimum diameter of colony of each test fungus was recorded. Mean value of the diameter of mycelial growth for each treatment were calculated. The inhibition percentage was calculated by using following formula (Grover & Moore, 1962):

$$\text{Percentage inhibition of mycelial growth} = \frac{Gc - Gt}{Gc} \times 100$$

where Gc = Fungal colony diameter in control plates, and Gt = Fungal colony diameter in plates amended with extracts. The experiments were conducted at the laboratory of Central Department of Botany, Tribhuvan University, Kathmandu, Nepal during July – August, 2014.

2.4 Data analysis

Data were analyzed using R program (version 2.15. 3) (R Core Team, 2015). Mean diameter of the mycelial growth was compared using one way ANOVA with post hoc (Tukey HSD) test.

3. Results

Root and litter extracts in concentration of 25% showed inhibitory activities against only *C. funicola*. Whereas, leaf extract at the same concentration inhibited mycelial growth of *A. alternata* and *C. funicola*. When the concentrations were increased to 50%, root extract showed inhibitory activity against *A. alternata, A. niger, C. funicola* and *T. harzianum*. Leaf extract at this concentration inhibited the mycelial growth of *A. alternata, C. funicola* and *F. oxysporum* (Figure 1).

The litter extract at 50% inhibited the growth of *C. funicola* and *F. oxysporum*. From all tested fungi, *A. alternata* and *C. fumicola* were inhibited the mycelial growth when using all type of extract (root, leaf and litter) in the concentration of 100%. However, *A. niger* was inhibited by 100% root extract only, *F. oxysporum* was inhibited by 100% leaf and litter extracts and *T. harzianum* was inhibited by 100% of root and leaf extracts (Figure 1).

The inhibition percentage was higher in leaf extract and increased with increasing concentration of both the leaf and root extracts. The leaf extract showed the highest percentage of inhibition to the mycelium growth of *A. alternata* in comparison to other extracts. In case of *C. funicola* the inhibition was the highest at high concentrations of litter extract (100%). It was interesting to note that the root extract was the most detrimental and inhibit the mycelial growth at higher concentrations in both *T. harzianum* and *A. niger*. Inhibitory pattern in *F. oxysporum* was similar to *A. alternata* and *C. funicola* but the percentage inhibition was comparatively lower (Figure 2). Radial growth of mycelium of tested fungi at different concen-trations of *A. adenophora* root, leaf and litter extracts has been shown in Figure 3.

4. Discussion

The results showed that the extracts from *A. adenophora* have antifungal property. It confirms that *A. adenophora* has influential role on ecologically very important components, the soil fungi, in their growth and development. Among selected test species, the *A. alternata* and *F. oxysporum* are common plant pathogens that cause damping off of seedlings in many plants (Agrios, 2005). Our results show that *A. alternata* is sensitive to all types of *A. adenophora* extracts but most sensitive to leaf extract because even at the low concentration (25%) of leaf extract, the growth was significantly reduced. In comparison to *A. alternata, F. oxysporum* showed resistivity to *A. adenophora* root extract but its growth was also checked at high concentrations of leaf and litter (50 and 100%) extracts. Consequently, it can be expected that *A. adenophora* limits the growth of *A. alternata* and *F. oxysporum* in its invaded sites through leaching from its aerial and underground parts. By this mechanism, *A. adenophora* could reduce mortality of its seedlings in invaded sites by limiting the

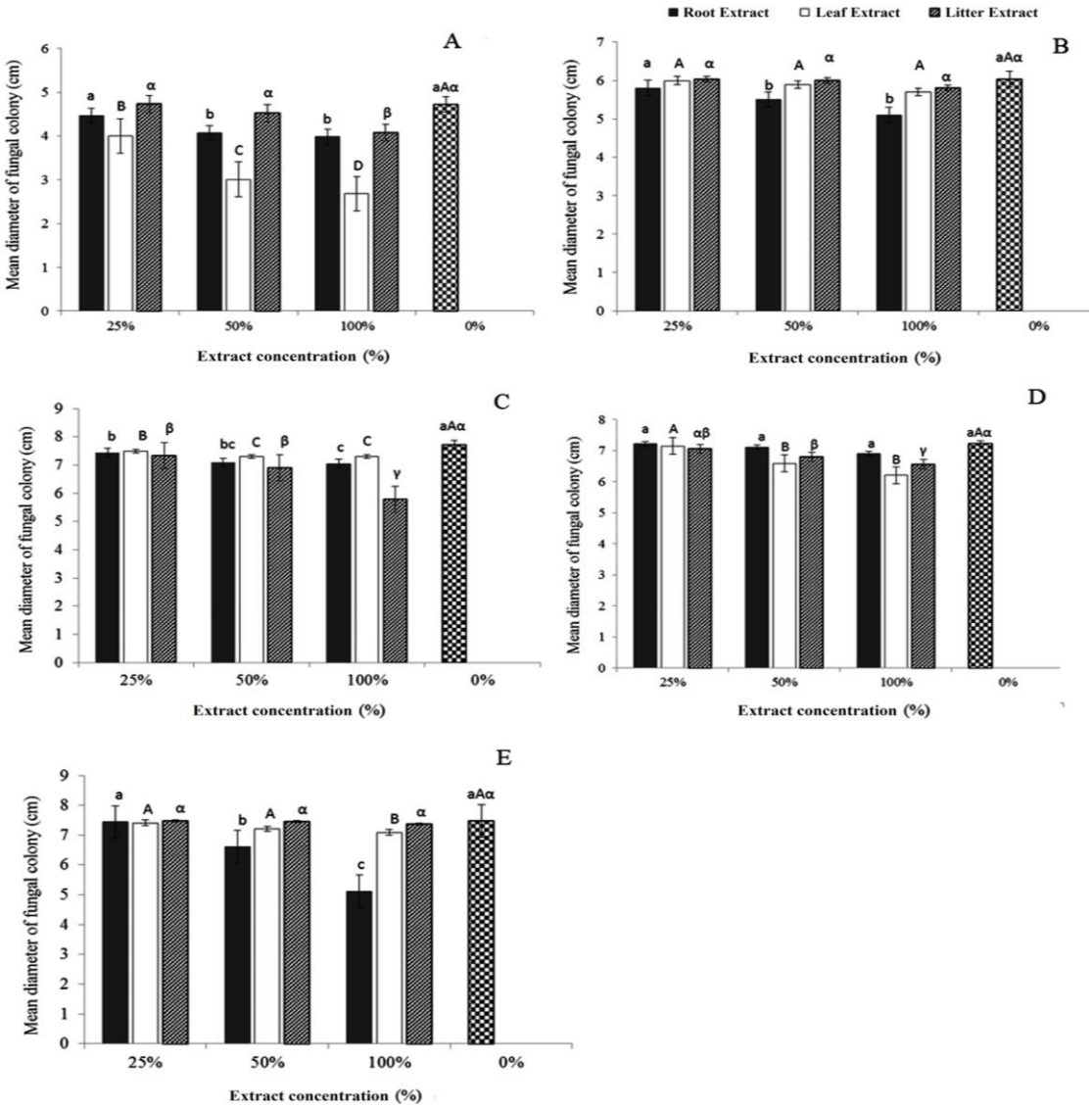

Figure 1. Mean radial growth of fungi; (A) *Alternaria alternata*, (B) *Aspergillus niger*, (C) *Chaetomium funicola*, (D) *Fusarium oxysporum*, and (E) *Trichoderma harzianum*. (Mean values sharing same letters are not significantly different, radial growth in root, leaf, and litter extracts are compared independently, thus assigned with different alphabets) (P = 0.01)

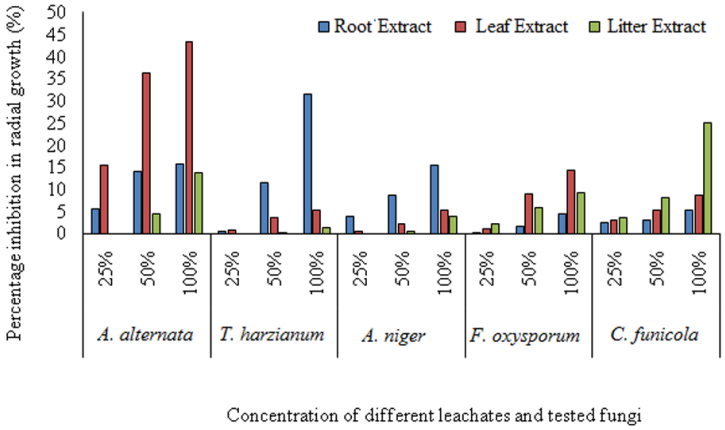

Figure 2. Percentage inhibition of radial growth of tested fungi by three different extracts.

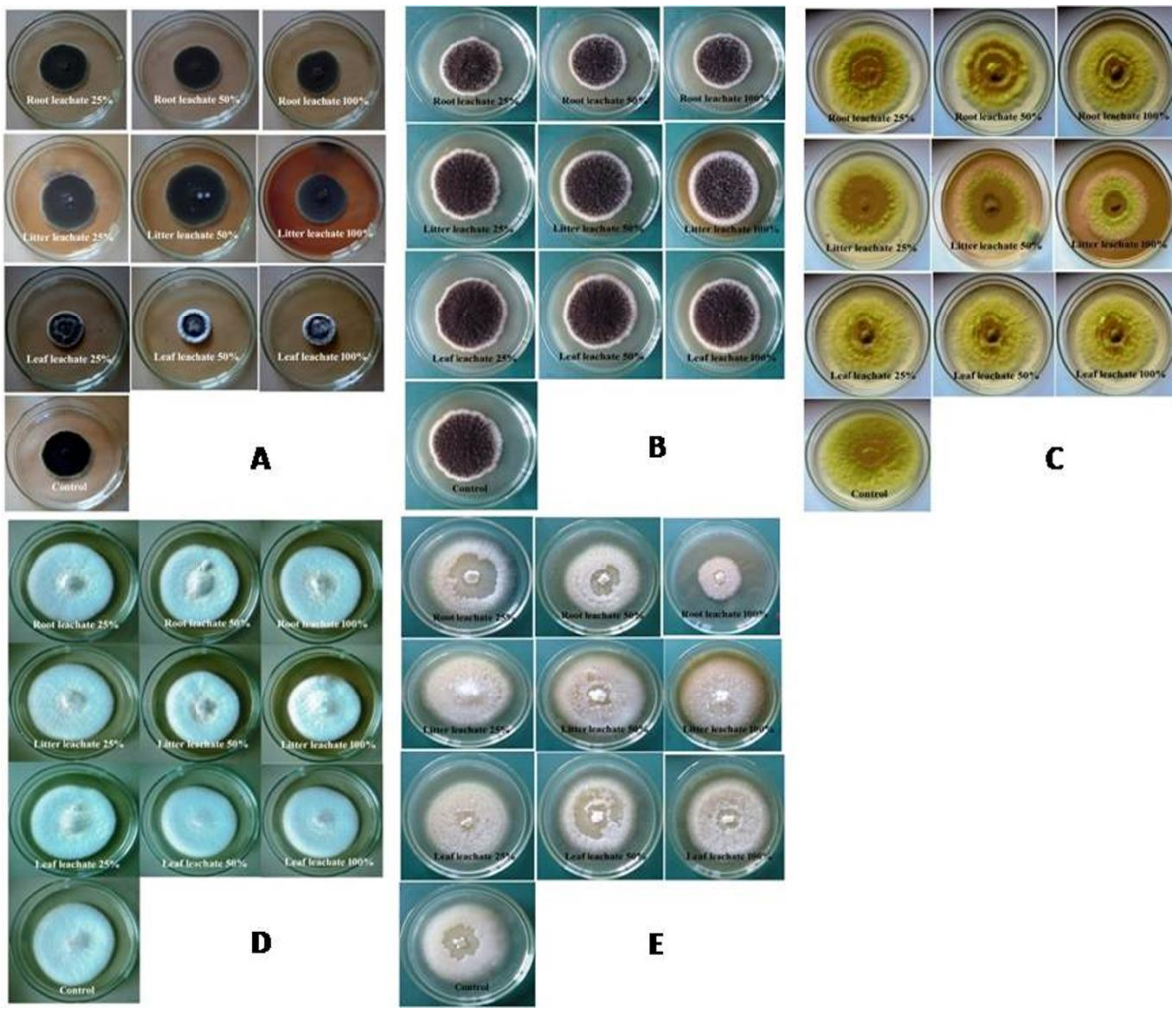

Figure 3. Culture plates showing radial growth of fungi (A) *Alternaria alternata,* (B) *Aspergillus niger,* (C) *Chaetomium funicola,* (D) *Fusarium oxysporum,* and (E) *Trichoderma harzianum.*

growth of pathogenic fungi cause damping off disease on seedlings. Therefore, limiting the growth of parasitic fungi could be one successful invasion mechanism adopted by *A. adenophora*.

On the other hand, *T. harzianum* and *A. niger* are common decomposers in natural ecosystems. They involve in decomposing large number of recalcitrant substances in forest floor (Thom & Raper, 1945). Eventhough, *T. harzianum* was resistant to litter extract and *A. niger* to leaf and litter extract, the extract from root inhibited their growth. Comparing to the role of these fungi and the inhibition to these fungi by *A. adenophora*, it could be anticipated that there is uncoupling of the nutrient cycling at invaded sites. It may results accumulation of nutrients at the invaded sites which could be beneficial for proliferation of invasive alien plants to utilize accumulated nutrients.

Although, the antibiosis property of *C. funicola* is not documented yet, other members of this genus possess antifungal and antibacterial properties (Brewer *et al.*, 1970; Istifadah *et al.*, 2006; Pietro *et al.*, 1992). As such inhibition to this genus by *A. adenophora* extract suggests that there is indirect effect of invasion on microbial population by changing microbial community composition.

It is interesting to note that not all types of *A. adenophora* extracts showed similar mode of inhibition to the mycelial growth of tested fungal species indicating that the growth of particular fungal species in the invaded soil may depends upon the type of extraction origin and concentration. It also indicates that different parts of *A. adenophora* can release the plant exudates having different allelochemicals which are specific to inhibit growth and development of particular fungus. This result was in accordance to our first hypothesis.

Invasive alien species are known to replace native species and negatively affecting native seed germination, growth and development (Inderjit *et al.*, 2011; Thapa *et al.*, 2016b). Mostly allelochemicals from aerial parts, specifically from leaves, have been investigated previously. Some of the metabolites, sesquiterpenes, triterpenoids, steroids, flavonoids, phenylpropanoids from *A. adenophora* are known to have insectcidal, antifungal and allelopathic effects on native plants

(Subba, & Kandel, 2012; Yongming *et al*., 2008; Zhang *et al.*, 2013). Zhang *et al*. (2013) have isolated quinic acid derivatives, 5-O-trans-O-coumaroylquinic acid methyl ester, chlorogenic acid methyl ester, macranthoin F and marcanthion G from aboveground part of *A. adenophora*. The compounds such as 1-naphthalenol, α-bisabolol, bornyl acetate, β-bisabolene, germacrene-D and α- phellandrene are the constituents of *A. adenophora* oil (Kurade *et al.*, 2010). In addition, epi-α-cadinol, α-phellandrene, δ-2-carene, γ-curcumene, camphene, epi- α-bisabolol, endo bornyl acetate, (E)-caryophyllene, β-bisabolene, α-trans bergamotene, germacrene, β-sesquiphellandrene and (E)- β-farnesene are considered as components of essential oil in *A. adenophora* (Pala-Paul *et al*., 2002) can also have adverse impacts on fungal growth and development. Possibly these chemicals might have inhibitory role in mycelial growth of ecologically important soil fungi.

Rainy season in Nepal and other south Asian regions is hot and humid which is favorable for optimum growth and activities of microorganisms. *A. adenophora* also favors this season for its luxuriant growth and sprouting new leaves. Therefore, *A. adenophora* is expected to release fungitoxic chemicals through extract in this season and may have inhibitory action against mycelial growth and development of ecologically beneficial fungi at invaded sites in nature. Previous works have revealed different mechanisms of *A. adenophora* invasion such as allelopathic inhibition to native species (Niu *et al.*, 2007), change in soil nutrients (Niu *et al*., 2007) and soil microbial community (Xue *et al.*, 2010).

Our results also support soil nutrient and soil microbial community change hypothesis (e.g. Balami *et al.* 2017) but the novel finding is that *A. adenophora* is able to control growth of fungi responsible for seedling mortality, decomposing recalcitrant substances and regulate bacterial community in the soil. Hence, we propose that these three mechanisms (i) ensuring of seedling survival (ii) accumulation of recalcitrant substances and (iii) changes in soil microbial population through antifungal activities are important mechanisms for successful invasion by *A. adenophora* in its non-native ranges. This evaluation of soil biotic changes by *A. adenophra* invasion paves the way for unraveling mechanism of this plant in introduced range. It will further advance our understanding on modification of soil biota during invasion of *A. adenophora*, and will helps us to know the probable effects on native plant nutrient acquisition strategies and survival.

Overall, extracts from different parts of *A. adenophora* influence growth and development of ecologically beneficial soil fungi. *A. adenophora* may get benefited by producing species specific chemicals to affect soil fungi. Degree of inhibition varied with extract and fungi type. Impact of *A. adenophora* on the soil fungi may bring changes in soil fungal diversity and their composition. Moreover, this mechanism could be a reason that affects soil characteristics, native diversity and ecosystem functioning during *A. adenophora* invasion.

Acknowledgements

Authors would like to express gratitude to Prof. Dr. P. K. Jha, former head, Central Department of Botany, Tribhuwan University (TU), Kathmandu, Nepal for providing laboratory facilities and various supports. We are thankful to Dr. C. Pokhrel, Central Department of Botany, TU for his valueble suggestions. We are grateful to the anonymous reviewers for invaluable suggestion and comments on the manuscript.

References

Agrios, G. N. (2005). *Plant pathology (Vol. 5).* Burlington, MA: Elsevier Academic Press.

Aneja, K. (2003). *Experiments in Microbiology, Plant Pathology and Biotechnology.* New Delhi, India: New Age International (P) Ltd.

Bajpai, D., & Inderjit. (2013). Impact of nitrogen availability and soil communities on biomass accumulation of an invasive species. *AoB Plants, 5*(ptr045), 1-9. doi: 10.1093/aobpla/plt045

Balami, S., Thapa, L. B., & Jha, S. K. (2017). Effect of invasive *Ageratina adenophora* on species richness and composition of saprotrophic and pathogenic soil fungi. *Biotropia*, *24*(3), 212-219.

Beest, M. T., Stevens, N., Olff, H., & Van der Putten, W. H. (2009). Plant soil feedback induces shifts in biomass allocation in the invasive plant *Chromolaena odorata*. *Journal of Ecology, 97*, 1281-1290. doi:10.1111/ j.1365-2745.2009.01574.x

Brewer, D., Jerram, W. A., Meiler, D., & Taylor, A. (1970). The toxicity of cochliodinol, an antibiotic metabolite of *Chaetomium* spp. *Canadian Journal of Microbiology*, *16*(6), 433-440. doi:10.1139/b06-083

Cantor, A., Hale, A., Aron, J., Traw, M. B., & Kalisz, S. (2011). Low allelochemicals concentrations detected in garlic mustard-invaded forest soils inhibit fungal growth and AMF spore germination. *Biological Invasion, 13,* 3015-3025. doi:10.1007/s10530-011-9986-x

Grover, R. K. & Moore, J. D. (1962). Toximetric studies of fungicide against brown rot organisms, *Sclerotinia fruticola* and *S. laxa*. *Phytopathology, 52*(9), 876–880.

Ha, T. J., Han, H. S., Jang, K. C., Jang, D. S., Cho, D. Y., Yang, M. S., & Lee, K. D. (2003). Antimicrobial activities of Sesquiterpene Lactones isolated from the flower of *Chrysanthemum coronarium* L. *Journal of the Korean Society for Applied Biological Chemistry*, *46*(3), 235-239.

He, W. M., Feng, Y., Ridenour, W. M., Thelen, G. C., Pollock, J. L., Diaconu, A., & Callaway, R. M. (20 09). Novel weapons and invasion: biogeographic differences in the competitive effects of *Centaurea maculosa* and its root exudate (+/-) catechin. *Oecologia*, *159*, 803-815. doi:10.1007/s00442-008-1234 -4

Inderjit, & Cahill, J. F. (2015). Linkages of plant–soil feedbacks and underlying invasion mechanisms. *AoB Plants, 7*(plv022), 1-8. doi:10.1093/aobpla/plv022

Inderjit, Evans, H., Crocoll, C., Bajpai, D., Kaur, R., Feng, Y. L., . . . Callaway, R. M. (2011). Volatile chemicals from leaf litter are associated with invasiveness of a Neotropical weed in Asia. *Ecology*, *92*(2), 316-324. doi:10.1890/10-0400.1

Istifadah, N., Jennifer, A., & McGee, P. A. (2006). Isolates of endophytic *Chaetomium* spp. inhibit the fungal pathogen *Pyrenophora tritici-repentis* in vitro. *Canadian Journal of Botany, 84*(7), 1148-1155. doi:10.1139/b06-083

King, R. M., & Robinson, H. (1970). Studies in the Eupatorieae (Compositae). XXXII. A new genus *Neocuatrecasia*. *Phytologia, 20*, 332-333.

Kundu, A., Saha, S., Walia, S., Shakil, N. A., Kumar, J., & Annapurna, K. (2013). Cadinene sesquiterpenes from *Eupatorium adenophorum* and their antifungal activity. *Journal of Environmental Science and Health, 48*(6), 516-522.

Kurade, N. P., Jaitak, V., Kaul, V. K., & Sharma, O. P. (20 10). Chemical composition and antibacterial activity of essential oils of *Lantana camara*, *Ageratum houstonianum* and *Eupatorium adenophorum*. *Pharmaceutical Biology, 48*(5), 539-544. doi:10.3109/13880200903193336

Lorenzo, P., Rodríguez-Echeverría, S., González, L., & Freitas, H. (2010). Effect of invasive *Acacia dealbata* Link on soil microorganisms as determined by PCR-DGGE. *Applied Soil Ecology, 44*(3), 245-251. doi:10.1016/j.apsoil.2010.01.001

Niu, H. B., Liu, W. X., & Wan, B. L. (2007). An invasive aster (*Ageratina adenophora*) invades and dominates forest understories in China: altered soil microbial communities facilitate the invader and inhibit natives. *Plant Soil, 294*, 73-85. doi:10.1007/s11 104-007-9230-8

Pala-Paul, J., Perez-Alonso, M. J., Velasco-Negueruela, A., & Sanz, J. (2002). Analysis by gas chromatography–mass spectrometry of the volatile components of *Ageratina adenophora* Spreng., growing in the Canary Islands. *Journal of Chromatography, 947*(2), 327-331. doi:10.1016/S0021-9673(02)00016-X

Pietro, A. D., Gut- Rella, M., Pachlatko, J. P., & Schwinn, F. J. (1992). Role of antibiotics produced by *Chaetomium* sp. in biocontrol of *Pythium ultimum*, a causal agent of Damping-off. *Phytopathology, 82*, 131-135.

Qiang, S. (1998). The history and status of the study on Crofton weed (*Eupatorium adenophorum* Spreng.), a worst worldwide weed. *Journal of Wuhan Botanical Research, 16*(4), 366–372.

Subba, B., & Kandel, C. (2012). Chemical composition and bioactivity of essential oil of *Ageratina adenophora* from Bhaktapur District of Nepal. *Journal of Nepal Chemical Society, 30*, 78-86. doi:10.3126/jncs.v30 i0.9350

Sun, X., Gao, C., & Guo, L. (2013). Changes in soil microbial community and enzyme activity along an exotic plant *Eupatorium adenophorum* invasion in a Chinese secondary forest. *Chinese Science Bulletin, 58*(33), 4101-4108. doi:10.1007/s11434-013-5955-3

Team, R. C. (2015). *R: A language and environment for statistical computing [Internet]*. Vienna, Austria: R Foundation for Statistical Computing.

Thapa, L. B., Kaewchumnong, K., Sinkkonen, A., & Sridith, K. (2016a). Plant communities and *Ageratina adenophora* invasion in lower montane vegetation, central Nepal. *International Journal of Ecology & Development™, 31*(2), 35-49.

Thapa, L. B., Kaewchumnong, K., Sinkkonen, A., & Sridith, K. (2016b). Impacts of invasive *Chromolaena odorata* on species richness, composition and seedling recruitment of *Shorea robusta* in a tropical Sal forest, Nepal. *Songklanakarin Journal of Science & Technology, 38*(6). doi:10.14456/sjst-psu.2016.86

Thapa, L. B., Kaewchumnong, K., Sinkkonen, A., & Sridith, K. (2017). Plant invasiveness and target plant density: high densities of native *Schima wallichii* seedlings reduce negative effects of invasive *Ageratina adenophora*. *Weed Research, 57*(2), 72-80. doi:10.1111/wre.12238

Thapa, L. B., Thapa, H., & Magar, B. G. (2015). Perception, trends and impacts of climate change in Kailali District, Far West Nepal. *International Journal of Environment, 4(4)*, 62-76.

Thapa, N., & Maharjan, M. (2014). Invasive alien species: Threats and challenges for biodiversity conservation - A case study of Annapurna Conservation Area, Nepal. *Proceedings of the International Conference on Invasive Alien Species Management, National Trust for Nature Conservation*, 18-22.

Thom, C., & Raper, K. B. (1945). *A manual of the Aspergilli*. Baltimore, MD: The Williams and Wilkins Company.

Tiwari, S., Siwakoti, M., Adhakari, B., & Subedi, K. (2005). *An inventory and assessment of invasive alien plant species of Nepal*. Kathmandu, Nepal: IUCN-The World Conservation Union.

Xue, L., Hong-Bang, N., Fang-Haoo, W., & Bo, L. (2010). Effects of leachates of the invasive plant, *Ageratina adenophora* (Sprengel) on soil microbial community. *Acta Ecologica Sinica, 30*(4), 196-200. doi:10. 1016/j.chnaes.2010.06.002

Yougming, L. I., Zhengyue, L. I., & Min, Y. E. (2008). The chemical composition and their bioactivities in the different parts of *Eupatorium adenophorum* Spreng. *Journal of Yunan Agricultural University, 23*(1), 42-46.

Zhang, M., Liu, W. X., Zheng, M. F., Xu, Q. L., Wan, F. H., Wang, J., & Tan, J. W. (2013). Bioactive quinic acid derivatives from *Ageratina adenophora*. *Molecules, 18*(11), 14096-14104. doi:10.3390/molecules18 1114096