



Research Article

Biocontrol of five invasive weeds of Meghalaya - A case study

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ABSTRACT: The state of Meghalaya, in North East India is endowed with a dense cover of natural forest. A vast majority of the forests are owned by communities who traditionally practice shifting cultivation. This has resulted in significant reduction of the original forest area leading to a drastic change in the floristic composition of the state. This is evident by the ubiquitous presence of various invasive weeds such as *Mikania micrantha*, *Chromolaena odorata*, *Ageratum conyzoides*, *Spilanthes paniculata* and *Spermocoe hispida*. A survey for natural fungal enemies against these weeds was carried out in the state with the objective of identifying potential biocontrol agents. A total of six pathogenic fungi were isolated from infected leaves of these target weeds. Of these, two fungi isolated from leaf spot and leaf necrosis disease of *Mikania micrantha* were identified as *Gliocladium roseum* and *Phomopsis* sp., respectively. The fungus isolated from *S. paniculata*, *C. odorata*, and *A. conyzoides* was *Fusarium solani* and from *Spermocoe hispida* it was *F. acuminatum*. Pathogenicity test carried out on target weeds and agricultural crops grown in Meghalaya (Maize, Chilli, Tomato, Rice and Ginger) showed that all the isolated fungi were found infecting the weeds from which they were isolated and also maize and tomato. They were found non pathogenic to seedlings of some economically important tree species of Meghalaya, viz., *Pinus kesiya*, *Magnolia champaca*, *Alnus nepalensis*, *Chukrasia tabularis*, *Exbucklandia populnea* and *Castanopsis indica*. Hence, these fungi can be tested in field condition on pilot scale, in forest areas but not in agricultural areas where maize or tomato is grown.

KEY WORDS: Biocontrol, Fungi, invasive, meghalaya, weeds

(Article chronicle: Received: 03-09-2018; Revised: 28-02-2019; Accepted: 30-04-2019)

INTRODUCTION

The state of Meghalaya, comprising of Khasi, Garo and Jaintia hills lying between 25°.00' and 26°.10' N and 89°.45' and 92°.45' E, is one of the richest state of India in terms of floral diversity and is endowed with a dense cover of natural forest comprising 76.45% of the total geographical area. A vast majority of the forests in Meghalaya are owned by clan/communities who traditionally practice shifting cultivation, as part of their socio-religious customs. About 70% of the state is forested, of which 9,496 km² (3,666sqm.) is a dense primary subtropical forest. The forests of Meghalaya are considered to be among the richest botanical habitats of Asia. These forests receive abundant rainfall and support a vast variety of floral faunal biodiversity. The forests of Meghalaya can broadly be grouped under the tropical type and the temperate type, mainly based on the altitude, rainfall and dominant species composition like tropical evergreen forests, tropical semi-evergreen forests, tropical moist forests, dry deciduous forests, grass and savannas, and temperate forests.

The alien plant invasion has been considered as a potential threat to the forest ecosystem in the tropics. It is estimated that as many as 50% of invasive species in general can be classified as ecologically harmful based on their actual impacts (Richardson *et al.*, 2000). Invasion of the species cause extensive effects on the habitats they invade, like impact on indigenous species diversity, soil nutrient composition, altering forest fire cycles and loss of productivity of invading ecosystems. It also becomes a threat to threatened plant species around the world (Pimentel *et al.*, 2005). Biological invasions possess a major threat to indigenous biological diversity. Invasive alien plants have caused extensive economic and ecological damage throughout the world. Therefore, the effects of biological invasion are increasingly being recognized for the role in degradation of biological diversity worldwide (Usher *et al.*, 1988; D' Antonio and Vitousek, 1992).

A rapid increase in human population, particularly the tribal populations who exclusively practice shifting cultivation (jhum), has resulted in the loss of forest cover and has paved

the way for the establishment of invasive weed species in areas where several unique native species previously existed. Major weed invasions change the natural diversity and balance of ecological communities. These changes threaten the survival of many plants and animals because the weeds compete with native plants for space, nutrients and sunlight. According to Rao and Sagar (2012), in Meghalaya, several native or naturalized grass species that have otherwise been eaten by cattle have become comparatively rare, for example *Bracharia mutica* Stapf., *B. repens* (L.) Gard. Et Hubb., *B. distachya* Stapf., *Paspalidium flabvidum* A. Camus, *Cyrococcum trigonum* A. Camus, *Panicum miliare* Lamk., etc. Haridasan and Rao (1985) have listed 45 species as being extremely rare, partly due to alterations in the natural habitat caused by invasive weeds.

The entire North Eastern region of India receives heavy rainfall and as a result, weeds and other undesirable plants grow luxuriantly in the cultivated and non-cultivated situations. Keeping the weeds under control has become a major problem in this region. Important invasive plants that have created havoc in a number of habitats in North Eastern India include terrestrial herbaceous weeds (e.g., *Ageratum conyzoides*), shrubs (e.g., *Chromolaena odorata*), vines (e.g., *Mikania micranthai*, and *Mimosa diplotricha*). These have entered the alien environment by one of two main pathways either intentionally to serve a human purpose or accidentally (through import of agriculture/horticulture material, human beings, ballast water etc). To get rid of those weed problems, the chemical herbicides were being used injudiciously which leads to resistance in weed species and also it affects the human and the environment directly or indirectly resulting in an ecological imbalance (Waghmare *et al.*, 2011). Further, herbicidal control cannot be recommended in natural forests because of the unique ecology and possible damage to the non target flora. Exploration of biocontrol agent for controlling the weed species could be an eco-friendly alternative to those hazardous chemicals. Thus, the present investigation was carried out to isolate and identify fungal pathogens naturally occurring on five target weeds, viz., *Mikania micrantha*, *Chromolaena odorata*, *Ageratum conyzoides*, *Spilanthes paniculata* and *Spermocoe hispida* in Meghalaya state and to assess the potential of these fungal pathogens for weed management.

MATERIALS AND METHODS

Survey for pathogenic mycobiota

The survey was carried out during 2016-2017 in different districts of Meghalaya to study the occurrence of disease incidence on the target weeds. The survey covered forest plantations and natural forest areas in all districts of the state.

Symptomatology

Different symptoms of the prevalent diseases occurring on the target weeds were noted and photographs were taken.

Isolation and identification of the causal organism

Diseased leaf samples were collected and brought to the laboratory wrapped on a brown paper bag. Fungi were isolated from the transition zone of healthy and diseased tissue of the sample. In isolating the fungi from samples, small pieces of about 2-5 mm of advancing zone of infection were cut and transferred to sterile Petri-dishes. The pieces were surface sterilized for 2-3 minutes in 4.0 per cent sodium hypochlorite (NaOCl). These surface sterilized cut pieces were transferred to the PDA (Potato Dextrose Agar) media in Petri-dishes. A trace amount of streptomycin was added to the media to inhibit the growth of bacterial population. The dishes were incubated at $24 \pm 1^\circ\text{C}$ and examined periodically for up to ten days. Identification of pure culture of the fungi from disease specimens were attempted on the basis of their cultural and morphological characters. Bits of mycelia growth obtained from the culture by inserting inoculating needle were mounted in lactophenol-cotton blue stains and sealed with nail polish to make them semi-permanent. The mount so prepared was observed under a compound microscope with a photographic attachment. The morphological features were recorded, measurements made and microphotographs were taken. For authentic identification or confirmation, the fungal cultures were referred to Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute (IARI), New Delhi.

Pathogenicity test

Tests for pathogenic association of the isolated fungi were made on target weeds, agricultural crops grown in Meghalaya (maize, chilli, tomato, rice and ginger) and also on seedlings of some economically important tree species of Meghalaya, viz., *Pinus kesiya*, *Magnolia champaca*, *Alnus nepalensis*, *Chukrasia tabularis*, *Exbucklandia populnea* and *Castanopsis indica*, and was confirmed through Koch's postulates. Seedlings of these plants were raised in earthen pots filled with sterile soil, under glass house conditions. Two mm diameter plugs of mycelial agar inoculums taken from ten days old fungal cultures with the help of sterile cork borer were used for inoculation. Control plants were inoculated only with sterilized agar bits. Both inoculated and controlled plants were kept covered with moistened polythene bags for 4 days. Observations were made periodically for symptom development, if any. Soon after the appearance of symptoms, the organism was re-isolated from artificially infected leaves and the isolate obtained was compared with the original culture for confirmation. Re-isolation from artificially infected plants was carried out on PDA.

Table 1. Study sites for occurrence of disease incidence on the invasive weeds of Meghalaya.

Districts	Sites	GPS Co-ordinates	Dominant Species
West Garo Hills	Ballonggre village	N-25°31'27.3"E-90°09'9.5"	<i>Chromolaena odorata</i>
	Gambakgre village	N 25°32.453' E 091°09.177"	<i>C. odorata</i>
	Jengjal	N-25°39'08.2"E-90°20'7.7"	<i>Spilanthes paniculata</i>
	Chinabat Village	N25°33.086' E090°22.849'	<i>Mikania micrantha</i>
	Rombagre Village	N25°33.375' E091°20.224'	<i>Ageratum conyzoides</i>
	Gambakgre Village	N25°32.453' E091°09.177'	<i>C. odorata</i>
South Garo Hills	Balonggre Village	N25°32'441" E090°09' 09.03"	<i>M. micrantha</i>
	Bandarigre	N25°23.227' & E090°15.399'	<i>C. odorata</i>
Ri-bhoi District	Dekudobagre	N-25°21'38.5"E-090°20'33.3"	<i>C. odorata</i>
	Umiam (U. C. C. Hills)	N25°39.450'E091°51.49.6'	<i>C. odorata, M. micrantha, Spilanthes paniculata</i> and <i>A. conyzoides</i> .
	(Umroi Village) Lumdiengngam	N25°38.249'E092°03.566"	<i>M. micrantha</i>
	Mawkhanu village	N25°38.249'E092°03.566"	<i>M. micrantha</i>
	Umtasor Range, Nongkhylllem Reserve Forest	N 25°50'.004"E 091°49'38.5"	<i>M. micrantha, S. paniculata, A. conyzoides</i> and <i>Spermacoce hispida</i>
West Jaintia Hills	Byrnihat, 15 th Mile	N 26°02'20.8"E091°52'.02.2'	<i>M. micrantha</i>
	8 th Mile Village	N25°28.191' E 092°17'24.8"	<i>M. micrantha</i>
	Sabhanous Village	N25°28'28.1" E092°14'51.2"	<i>A. conyzoides, S. paniculata</i>
	Khliekhtyrshi Village	N25°28'38.7" E092°10'39.0"	Large scale of <i>A. conyzoides</i> notice but no disease was found.
East Jaintia Hills	Mookyndur Village	N 25°31'57.5" E092°07'12.9"	<i>M. micrantha</i>
	Sutnga village (Site A)	N25°22'45.2" E 092°26'57.8"	<i>M. micrantha</i>
	Sutnga village (Site B)	N25°22'29.9" E 092°26'24.2"	<i>M. micrantha, A. conyzoides</i>
	TongsiangNong-Khlteh Villag	N25°19'37.9"E092°30'08.1'	<i>M. micrantha</i>
	Dkhiah Village	N25°22'01.4"E092°21'0.13"	<i>Spilanthes paniculata</i>
	Sohshrieh Village	N 25°26'6.3"E 092°17'35.4"	<i>conyzoides</i>
	SonapurNarpoo Reserve Forest, Jowai	N 25°05' 28.7" E 092°21'25.0"	<i>M. micrantha</i>
East Khasi Hills	3 rd Mile	N25°30.359'E091°40.965'	<i>A. conyzoides</i> were noticed; but no disease was found.
	Ichamati	N25°10'.04.4" E 091°41'18.2"	<i>M. micrantha</i>
	Shellapunji	N25°10'86.3"E091°38'026"	<i>M. micrantha, C. odorata, and A. conyzoides</i>
	Laitkynsew Village	N25°13'11.5"E091°40.04"	<i>S. paniculata</i>
	Upper Shillong, Sangmei Terrestrial Division	N 25°32' 22.4" E091°50'54.3	<i>M. micrantha</i>
	Sohra Forest Range	N 25°13.029'E 091°43.405'	No weeds found in these places
	Thangkhang Village	N25°12.99.3"E91°43'85.4"	
	Mawngap	N25°28.88'E91°45.407"	
East Garo Hills	Rongrengre Reserve	N25°32'46.3" E090°09' 04.1"	<i>C. odorata</i>
	Khocshinegsak	N25°30'65.1"E090°06.9"	<i>C. odorata</i>
	Chisobibra Village	N25°30.655' E090°08.253'	<i>C. odorata</i>
	Nenkharra Village	N25°30.356' E090°49.959'	<i>M. micrantha</i>
North Garo Hills	Wagachi	N25°49' 29.3" E 090°47'10.7"	<i>M. micrantha</i>
	Dangkhong area	N25°51' 09.2" E 090°47'08.8"	<i>M. micrantha, C. odorata, and A. conyzoides</i> .
	Soinang (Upper)	N25°51'51.3" E090°37'025.6"	<i>C. odorata</i>
	Thapa Darengri	N25°50'07.0"E091°35'55.8"	<i>M. micrantha</i>
	Sapicol	N25°48 '21.7" E 090°37 '07.1"	<i>M. micrantha</i>
South West Khasi Hills	Mawlyngroh	N25°14.55.6' E 091°24.58.8',	<i>M. micrantha</i>
	Wahkaji	N25°22.169' E 091°15. 756',	<i>A. conyzoides</i>
	Phottdei Village	N25°23.352"E 091°23. 190",	No species found

	Sakwang Village	N25°23.374' E 091°28. 842',	<i>A. conyzoides</i>
	Mawsaw Dom Shrah	N25°25.425 ' E 091°22. 513',	No species found
	Mawlangbang Village	N25°22.964' E 091°23. 830',	<i>A. conyzoides</i>
West Khasi Hills	Nongstein	N25°30.610' E 091°13. 705',	<i>A. conyzoides</i>
	Wahlulu	N25°32.222' E 091°40. 773',	<i>A. conyzoides</i>
	Nongtraw Village	N25°29.679' E 091°08. 558',	<i>A. conyzoides, M. micrantha</i>
	Mairang Langstiehrim	N25°34.281' E 091°38. 121',	No species found
	Riangdo	N 25°40.111', E 091°03.940'	<i>M. micrantha</i>
South West Garo Hills	Balnangre	N25°35'11.4"E90°02'F35.2"	<i>C. odorata</i>
	Wakka Akonggre	N25°30.055' E090°08.253'	<i>C. odorata</i>

***In vivo* evaluation of isolated fungi at Rain Forest Research Institute (RFRI), Jorhat campus**

Four different fungi were used for *in vivo* study at RFRI, Jorhat campus. The fungi were mass cultured in PDB (Potato Dextrose Broth) media. The PDB along with the full grown mycelial mat was then mixed with sterile water to prepare a final concentration of 3 per cent spray solution. Jaggery @ 10 per cent was also mixed with the spray solution so as to serve as a sticking agent. Spraying of biocontrol agent was done at an interval of 10 days on target weeds, viz., *Mikania micrantha*, *Chromolaena odorata* and *Spilanthes paniculata*. Control plants were maintained by spraying sterile distilled water.

Observations

Observations on mortality of the target weed species were recorded at an interval of 7 days after each spray and disease incidence was recorded. Mean increase or decrease of the disease development after the treatment was calculated in comparison to control.

RESULTS AND DISCUSSION

The survey indicated the ubiquitous presence of *Mikania micrantha* and *Chromolaena odorata* in the state whereas *Ageratum conyzoides* and *Spilanthes paniculata* were present only in a few districts. *Spermacoce hispida* was found only in Ri-Bhoi district.

A total of six pathogenic fungi were isolated from infected leaves of these target weeds. Of these, two fungi isolated from leaf spot and leaf necrosis disease of *M. micrantha* were identified as *Gliocladium roseum* and *Phomopsis sp.* respectively. The fungi isolated from *S. paniculata*, *C. odorata*, *A. conyzoides* were identified as *Fusarium solani* and from *S. hispida* as *F. acuminatum*.

Studies on symptomatology

***Mikania micrantha* leaf spot**

The most common disease found on *M. micrantha* was leaf spot. Initial symptoms appeared as minute pale brown

spots which enlarge to form irregular spots with grey centers and dark brown margins. This disease is widespread in Meghalaya.

The fungus isolated from leaf spot of *M. micrantha* was identified as *Gliocladium roseum* (ITCC No. 10,720.18). The fungus grows rapidly in culture producing spreading colonies with a cottonlike texture, covering a petri-dish in 1 week. The colonies are white and cream- like. The species produces hyphae, conidiophores and conidia borne from hyaline phialides. The conidiophores are erect, dense and have a brush – like structure which produce tapering, slimy phialides.

Leaf necrosis

Initial symptoms appeared as pale brown spot on the leaf lamina. Later on, the spots enlarged in size and coalesced to form large necrotic areas. The fungus isolated from leaf necrosis of *M. micrantha* was identified as *Phomopsis sp.* (ITCC No. 10.721.18). The colony was initially white in colour, cottony growth with straight mycelium. Later, colony became greyish in colour. Mycelia were septate. Pycnidia were dark, ostiolate, immersed, erumpent, almost globose. Conidiophores were simple. Conidia hyaline, 1 celled, of two types, ovoid to fusoid conidia and filiform, curved or bent stylospores (beta conidia).

***Chromolaena odorata* leaf spot**

Symptoms appeared as irregular, brown to dark brown spots of various sizes scattered all over the leaf lamina. Both young and older leaves were found infected. The fungus isolated from *C. odorata* was identified *Fusarium solani* (ITCC No. 10,722.18). The colonies are usually fast growing, pale or bright coloured with or without a cottony aerial mycelium. Most of the *Fusarium sp.* produced both macro and micro conidia from slender and simple phialides. Macro conidia hyaline, two to several cell, fusiform to sickle shaped conidia, mostly with an elongated apical cell and pedicellate basal cell. Micro conidia one or two celled, hyaline, pyriform-fusiform to ovoid, straight. Chlamydo spore may be present or absent.

Spilanthes paniculata* and *Ageratum conyzoides

On *Spilanthes paniculata* symptoms appeared as brown necrotic lesion starting from the tip and spreading downward through the leaf margin. Distribution of the disease is very restricted and collected only from *S. paniculata* grown in swampy areas. On *Ageratum conyzoides* initial symptoms appeared as irregular brown necrotic lesion on the leaf margin. Later on, enlarged in size and covered the entire leaf surface.

The fungus isolated from *S. paniculata*, *A. conyzoides* (ITCC No. 10,723.18, 10,724.18) was identified as *Fusarium solani*. So, the characteristics are same as the above.

Spermacoce hispida

The fungus isolated from *Spermacoce hispida* was identified as *Fusarium acuminatum* (ITCC No. 10,725.18). The colonies are usually fast growing, bright coloured with or without a cottony aerial mycelium. Most of the *Fusarium sp.* produced both macro and micro conidia. Oval to kidney shaped micro conidia and sickle-shaped, thin walled and delicate macroconidia. Micro conidia produced in false heads on short monophialides. Chlamydospore may be present or absent.

Results of pathogenicity test revealed that all the isolated fungi were found infecting the weeds from which they were isolated, whereas only maize and tomato were found infected.

Pathogenicity test carried out on seedlings of some economically important tree species of Meghalaya, viz., *Pinus kesiya*, *Magnolia champaca*, *Alnus nepalensis*, *Chukrasia tabularis*, *Exbucklandia populnea* and *Castanopsis indica* revealed that the isolated fungi were not infecting the seedlings.

In vivo application of isolated fungus on *Mikania micrantha* (leaf necrosis and leaf spot), *Chromolaena odorata* and *Spilanthes paniculata* was carried out in RFRI campus.

In the present investigation, the two fungal pathogens, viz., *Gliocladium roseum* and *Phomopsis sp.* isolated from *M. micrantha* were different from those fungal pathogens reported from Kerala (Sreenivasan and Sankaran, 2001) and from North East India (Gogoi, 2001). Hence, these two fungal pathogens can be considered as new records on *M. micrantha* from India. Further, the fungus *Fusarium solani* is also reported to be isolated for the first time from the *C. odorata*, *S. paniculata* and *A. conyzoides*. Chetti *et al.*, (2001) reported three different fungi, viz., *Colletotrichum gloeosporioides*, *Alternaria alternata* and *Aureobasidium pullulans* to cause diseases on chromolaena. The fungal

pathogen *F. acuminatum* isolated from *Spermacoce hispida* might be considered as an opportunistic fungus as it did not cause any serious damage to the host.

Kaur *et al.*, (2014) also reported the phytotoxicity of *Fusarium solani* on *Parthenium spp.* which caused severe chlorosis, necrosis and complete death of the leaves and is similar to our findings.

Phomopsis spp. was also found to be effective in killing the target weeds. Plant mortality occurs more quickly in fungus treated plot as compared to the control which is similar with the findings of Roskopff *et al.* (2000) where they found 73% mortality of *Amaranthus lividus* with a single application of *Phomopsis amaranthicola*, while within four weeks the mortality rate increased to 95% after the second spray. In the present investigation, spraying of fungal spore suspension of *Fusarium solani* on chromolaena infected only the leaves and this finding is similar to that of (Chetti *et al.*, 2001), who also reported that *C. gloeosporioides* and *A. alternata* could only infect the leaves.

The present study indicates that the different isolated fungus seems to offer great potential for development and exploitation as effective biocontrol agents against the target weed species in large scale under field condition. Field evaluation of biological effectiveness is one of the most important components of a complete evaluation of a pathogen's weed-control potential. Although a pathogen may perform effectively in greenhouse or growth-chamber experiments, its use in the field is often characterized by variable levels of effectiveness against the target weed. In addition, most synthetic and natural pesticides are susceptible to ineffectiveness due to resistance buildup in different weed species. Thus, the only viable solution for the future is integrated management of weed. The economic benefits and reduced social costs of these systems present a logical answer to the weed control problem.

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