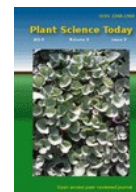




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Research Article

First evidences for induced pseudo-viviparous germination in *Ageratina adenophora* (Crofton weed), a common alien weed of Darjeeling Himalaya, India

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Abstract

In the present investigation, an autecological study has been carried out for *Ageratina adenophora* (Spreng.) R.M. King & H. Rob., a common aggressive alien weed found in dense patches at different localities of Darjeeling Himalaya, West Bengal, India. Single generation colony, derived via induced pseudo-viviparous germination, outnumbers the associated species by its aggressiveness and allelopathic potentiality. The cypselsae (fruits) mature during full monsoon and being laden with water droplets shed their deciduous calyx (pappus). The later thus cannot disperse away from the head (capitulum) by effective parachute mechanism. Very frequently, many of the cypselsae get germinated with two minute paracotyledons on the receptacle that still attached with the mother plant by decaying receptacle stalk. Entire head with cluster of seedlings, becoming heavy by monsoon shower, drops down on the lower wet substratum. It is the beginning of the colony formation. Vigorous growth and allelopathic potentiality of the species decreases the establishment, growth and density of other plant species in that area facilitating procurement of more space and nutrients for the individuals by itself, leading to make a dense colony with very high Importance Value Index (IVI). All these features may be considered as an adaptation to maintain its invasiveness and dominance over the surrounding species.

Keywords

Ageratina adenophora; invasive plants; pseudo-vivipary; allelopathy; Importance Value Index (IVI)

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Introduction

Ageratina adenophora (Spreng.) R.M. King & H. Rob., also known as *Eupatorium adenophorum* Spreng. (1826), belongs to the family Asteraceae. The plant is commonly known as crofton weed or sticky eupatorium, being other local common names as sticky snakeroot, Mexican devil, and cypress weed in USA, New Zealand and India, respectively. It is native to central Mexico and

thereby introduced in many other countries as an ornamental plant. Subsequently, this new comer has established and spread over the regions and became an invasive species. *Ageratina adenophora* was introduced in India after 1498 (Biswas, 1934). At present, it is an important alien species in Indian flora. The plant rated as class-iv noxious weed under NSW (New South Wales) noxious weed act, 1993.

Ageratina adenophora is a perennial under shrub, growing up to about 1-2 meters with opposite trowel-shaped serrate leaves (6-10 cm x 3-6 cm) having three primary veins. Small creamy white composite flowers bloom in monsoon on characteristic capitulum (head) of 0.3 cm to 0.5 cm in diameter. The heads develop in terminal clusters to the branches. The fruits (cypsela) are small, with simple, deciduous pappus.

The name 'Crofton weed' is used for the species as it significantly contains essential oil in its aerial parts (Qu *et al.*, 2011), and its dense patches over a large area prevent soil erosion (Baniya and Bhattarai, 1984). In the present work we noticed induced pseudo-vivipary and single generation colony formation, in addition to allelopathic potentiality leading to its aggressiveness and dominance.

Vivipary is a common reproductive feature of mangroves. Their seeds do not enter dormancy and germinate protruding their radicles whilst attached to the maternal plant. Some plants like members of the Rhizophoraceae show continuing embryo growth prior to dispersal, like vivipary. Here, embryo growth begins with the protrusion of a cotyledon body from the seed before dispersal. Radicle protrudes on the substratum only after shedding. The process is characterized as "semi-vivipary" (Ismail, 2010). Pseudo-vivipary is the feature of producing propagules like plantlets in an inflorescence, instead of seeds whether all other parts of the plant appear normal (Elmqvist and Cox, 1996). This interesting feature mostly induced by habitat condition and vary with area to area, species to species or season to season (Gordon-Gray *et al.*, 2009), as previously reported in some members belonging to Cyperaceae (Gordon-Gray *et al.*, 2009) and Poaceae (Vega and Rugolo-de-Agrasar, 2006; Milton *et al.*, 2008; Kuzmanović *et al.*, 2012).

Among the Eastern Himalayan angiosperms, vivipary has been reported in very few species that include *Sechium edule* (Jacq.) Sw. (chayote) of Cucurbitaceae (Aung *et al.*, 1990), and *Trifolium repens* L. (white clover) of Fabaceae (Majumdar *et al.*, 2004). Like vivipary, 'pseudo-vivipary' is a mode of ecophysiological adaptation, developing a continue reproduction to escape dormancy for the establishment of new generation in their prevailing habitat with excessively wet or saline marshy soil. In the present work the characteristic of pseudo-vivipary has been found in the non-mangrove plant *A. adenophora* of Asteraceae as an adaptive advanced reproductive trait for aggressiveness via single colony formation that may be useful for greater possibilities of survival and dominance of the species.

Some plants exert allelopathic influence (Gawronska *et al.*, 2006; Inderjit *et al.*, 2003; Putnam *et al.*, 1978; Reigosa *et al.*, 2006) by secreting a number of secondary metabolites which have inhibitory effect on the growth and

development of other plants, microorganisms and animals (Calera *et al.*, 1995). Such allelopathic potentiality has been reported in *A. adenophora* by Rai and Tripathi in 1981 together with many other researchers thereafter.

In the present work, allelopathic potentiality of the species has been studied in connection with the quantitative analysis of *A. adenophora* dominated discrete plant communities by quadrat method. High density of the species in an area enhances the concentration of the released allelochemicals that in turn enhances the elimination of non-self-species individuals.

Materials and Methods

Relative dominance study by community analysis

The present autecological study on *A. adenophora* has been carried out at different altitudinal zones of the Eastern Himalayan hill station Darjeeling (2,042 m AMSL) and its adjoining areas like Tiger hill (2,590 m AMSL), Lebong (1,661 m AMSL), Kalimpong (1,250 m AMSL), Lava (2,138 m AMSL), Rishop (2,138 m AMSL) and Neora valley (2,360 m AMSL) during monsoon and long post monsoon seasons. Observations were continued through successive five years (July 2010 to June 2015). The localities of study include roadsides, forest boundaries, and undisturbed slopes of hills among others. The annual mean temperature of the regions with cold temperate climate varied between 5°C to 16.5°C and the average annual precipitation is about 309.2 cm. During monsoon (June to August) average temperature ranged between 13°C to 19°C. It rained increasingly and the weather remained foggy or misty for most of the times. This was the period when humidity in Darjeeling was at its highest levels and it ranged between 93-96% of saturation. Average numbers of rain fall and rainy days during monsoon months in Darjeeling are June 2.06 cm (24.1 days), July 2.5 cm (32.0 days) and August 2.44 cm (26.6 days).

Distribution and dominance of the plant in its community was analyzed following the quadrat method (Priestley, 1913) with some modifications. In the current investigation sampling was done from ten different areas of the above mentioned localities. Sampling spots were selected on the basis of croftonweed invasion and sizes of the sampling quadrats were determined as 40 cm x 40 cm following species area curve i.e. manageable minimum size of the sampling unit covering maximum number of species in the spots under study. At each study site, four sampling quadrats were laid down. Number and basal diameter of each individual of *A. adenophora* and its associated species were noted down. The collected data of the four quadrats of each study site were combined together for the convenience of calculation. The sampling quadrats Qi+Qii+Qiii+Qiv had been taken as Q1 for site 1



Figure 1: Image part of a flowering plant part of *Ageratina adenophora* (Spreng.) R.M. King & H.Rob.

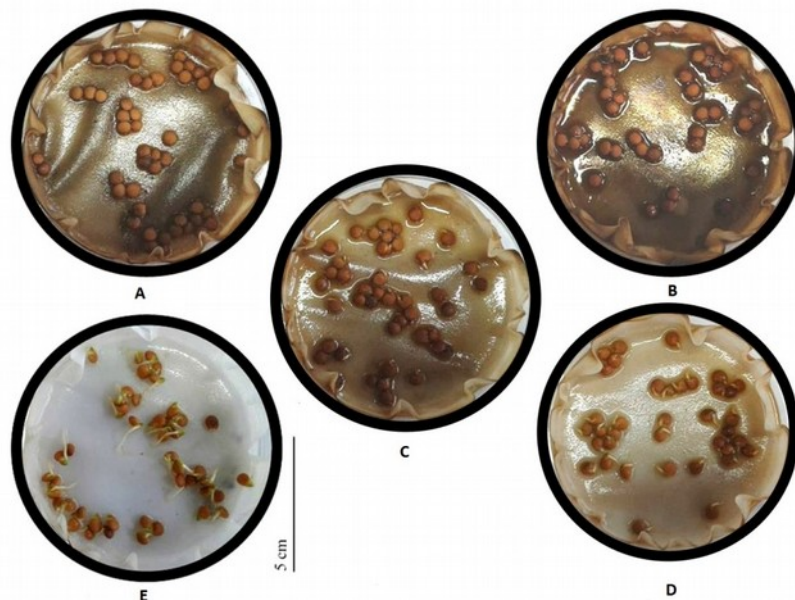


Figure 2: Allelopathic effect of varying concentrated tissue extract [A=1:5; B=1:10; C=1:20; and D=1:40 (tissue extract: water, respectively) compared to E=Control] on seed germination of lentil seeds

and followed accordingly up to Q10. From the field data of frequency, density, abundance, basal area, relative frequency, relative density, relative dominance, importance value index (IVI) and association index were calculated to have a quantitative structure of *A. adenophora* membered plant communities.

Allelopathic potentiality

In relevance of the work, an experiment on allelopathic potentiality of *A. adenophora* was carried out by using aqueous extract from the aerial parts on *in vitro* seed germination, and seedling growth inhibition of lentil (*Lens culinaris* Medik.), rice (*Oryza sativa* L.) and mustard (*Brassica napus* L.) seeds following the

conventional method (Rai and Tripathi, 1981). Different concentration gradients of the plant extract were applied on the seeds under the experiment, and allowed to germinate on petri dishes with soaked blotting paper by maintaining moisture content. The biological assay was carried out in triplicate sets along with control sets. It was checked on regular basis in relation to the percentage of germination and the lengths of newly born radicle and plumule of the seedlings.

Microscopic observation

In the search of the structural specialty of the cypsela, microscopic studies from mature specimens were also carried out with the help of a binocular dissecting stereo microscope (Olympus).

The process of imaging capitulation of the cypsela and pappus was done with the aid of a digital camera (Olympus ST-500UZ).

Observation of induced pseudo-viviparous germination and colony formation

Pseudo-viviparous germination of the studied members have been evidenced by still photography captured during *in vivo* cypsela-seed germination that take place on the head receptacle still attached with mother plant and the subsequent formation of seedling colonies after falling on the lower substratum whatever on neighboring plant's leaf or the soil beneath.

Collection and documentation of different stages of seedling development

Seedlings with different stages of development (two para-cotyledons stage to eight leaves stages) were collected from the germinating seeds on the head as well as from the soil substratum. The collected seedlings were dried through paper pressing, mounted sequentially on herbarium sheets and photographed.

Results and Discussion

Community analysis

The data collected from the studied areas by quadrat sampling, deliberately in *A. adenophora* (Figure 1) inhabited areas, shows its remarkably high dominance over other associated plant species (Table 1). In average, this species covered almost 3/4th of its habitat itself. Among the 13 species found in the *A. adenophora* (relative frequency 28.57%, see Table 1) dominant colonies the relative frequency (%) was higher in case of *Pilea umbrosa* Bl. (14.28), *Drymaria villosa* Cham. & Schltld. (8.57) and *Hypoestes triflora* (Forssk.) Roem. & Schult. (8.57) (Table 1). Similarly, importance value index (IVI) was highest in case of *A. adenophora* (198.38) among all members in the studied quadrats made a strong point to conclude the species as a notorious which dominate over the community and outnumbers the other members present in the colony where it belongs. Moreover, it was found that *A. adenophora* shows maximum association with *P. umbrosa* and a moderate level with *D. villosa* and *H. triflora* (see Table 1).

Invasive species are described by several aspects as they have been introduced by human activity into ecosystems where they are not native, and they have established populations of advantageous fast growing and self-reproducing, causing significant changes in pre-existing ecosystems (Richardson, 1994). Likewise, the increasing frequency, spatial patterns or scale of disturbances do lead to faster replacement of native species by exotic species (Yan *et al.*, 2001). Massive invasion and spreading over colony is due to also typically allelopathic reasons (Chettri, 1986;

Rai and Tripathi, 1982). From field observation and all available information it is clear that *A. adenophora* follows the typical trends of an invasive weed. It mainly found in hilly temperate and subtropical areas that conforms to the statement that dominance of *Ageratina* species has occurred in transitional zones with adequate moisture (Kunwar, 2000). Invasive effect of this plant has become complex through variation in reproductive strategy and growth mode.

Allelopathic potentiality

Allelopathic potentiality test using aqueous extract of aerial parts of the weed showed significant effect on germination and seedling growth of three common field crops: lentil (*L. culinaris*), rice (*O. sativa*) and mustard (*B. napus*) seeds (Table 2). It was observed that the lentil seed germination and growth of plumule and radicle was adversely affected (Figure 2). Germination was not started at all in the sets of concentrated plant extract (weed tissue and water proportion 1:5 and 1:10). After certain level of dilution, germination started but both the radicle and plumule growth were slow. The growth rate was gradually increased with further dilution of the extract and was maximum in case of control set that was devoid of the tissue extract. In case of rice and mustard germination was totally absent in presence of a minimum aqueous extract (result not shown). Up to six days of observation a single radicle was not found to be emerged from these two species seeds under the experiment in all replica sets except the control ones (result not shown).

From the experimental results it can be suggested that *A. adenophora* has sufficient potentiality to suppress growth of the cultivated crop species, also in addition to the wild herbs it dominate over colony. Same observation on another species was found and reported by Tripathi *et al.* (1981) where amongst the test species *T. repens* suffers the most. The different degree of effect by varying concentrated extract, inhibiting seed germination and the species specificity at suppression level, has been reflected in the present study and seems to be in congruence with the earlier report by Akhtar *et al.*, 1978 and Whittaker *et al.*, 1971. These effects are due to certain chemicals called allelochemicals, generally found in the aerial parts of different plant species. After abscission of leaves and other parts of the plant falling under and around its canopy thereby decomposed and the chemicals incorporated into soil. Same action also occurred through rainfall which dissolves some amount of such chemicals from shoot canopy of the plant and mix into the soil. All these events ultimately increases the allelochemicals in the underneath soil. Presence of this allelochemicals affects the germination and growth of neighboring plants

Table 1: Results of community analysis by Quadrat method and determination of Importance Value Index & Association Index

Species	R ¹	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	n ²	BA ³	Total BA	Total Q ⁴	f ⁵	Density	Abundance	Relative frequency (%)	Relative density (%)	Relative dominance (%)	IVI ⁶	Association Index ⁷
<i>Ageratina adenophora</i>	4	23	30	15	25	27	11	42	17	33	21	244	50.29	12270.76	10	100	24.4	24.4	28.57	75.78	94.03	198.38	
<i>Pilea umbrosa</i>	1.8	3	-	-	4	-	7	-	2	-	3	19	10.18	193.42	5	50	1.9	3.8	14.28	5.9	1.48	21.66	0.5
<i>Drymaria villosa</i>	0.7	2	-	1	-	-	-	-	5	-	-	8	1.54	12.32	3	30	0.8	2.7	8.57	2.48	0.09	11.14	0.3
<i>Calamintha umbrosa</i>	2	1	-	-	-	-	-	3	-	-	-	4	12.57	50.28	2	20	0.4	2.0	5.71	1.24	0.39	7.34	0.2
<i>Hypoestes triflora</i>	1.5	-	2	-	1	-	-	-	-	5	-	8	7.07	56.56	3	30	0.8	2.7	8.57	2.48	0.43	11.48	0.3
<i>Trifolium repens</i>	2	-	5	-	-	-	-	-	-	-	-	5	12.57	62.85	1	10	0.5	5.0	2.86	1.55	0.48	4.89	0.1
<i>Polygonum runcinatum</i>	1.6	-	-	2	-	-	-	-	-	-	-	2	8.05	16.1	1	10	0.2	2.0	2.86	0.62	0.12	3.6	0.1
<i>Calceolaria mexicana</i>	2	-	-	-	7	-	-	-	-	-	-	7	12.57	87.99	1	10	0.7	7.0	2.86	2.19	0.67	5.72	0.1
<i>Achyranthes bidentata</i>	2.3	-	-	-	-	4	-	-	-	2	-	6	16.62	99.72	2	20	0.6	3.0	5.71	1.86	0.76	8.33	0.2
<i>Persicaria amplicaulis</i>	2.2	-	-	-	-	-	3	-	-	-	1	4	15.21	60.84	2	20	0.4	2.0	5.71	1.24	0.51	7.46	0.2
<i>Urtica dioica</i>	1.5	-	-	-	-	-	-	1	-	-	2	3	7.07	21.21	2	20	0.3	1.5	5.71	0.93	0.16	6.8	0.2
<i>Lindenbergia grandiflora</i>	1.3	-	-	-	-	-	-	-	1	-	2	1	5.31	5.31	2	10	0.1	1.0	2.86	0.31	0.04	3.21	0.2
<i>Genanium nepalense</i>	1.6	-	-	-	-	-	-	-	-	7	-	7	8.05	56.35	1	10	0.7	7.0	2.86	2.19	0.43	5.48	0.1
<i>Sonchus arvensis</i>	3	-	-	-	-	-	-	-	-	-	2	2	28.29	56.58	1	10	0.2	2.0	2.86	0.62	0.43	3.91	0.1
Total												322				350							

¹ Av: Radius (mm) ² Total No. (n) ³ Basal Area (BA) ⁴ Total Qs possessing the spp ⁵ Frequency (%) ⁶ Importance Value Index (IVI) ⁷ Association Index of *Ageratina* (x) with other species (y)



Figure 3: Cypselum under microscope with deciduous pappus showing different stages of shedding (a-d)



Figure 4: The single generation colony inhabited by *Ageratina adenophora*

Table 2: Allelopathic potentiality and seed germination inhibition

Concentration of tissue extract	Length of growing radicle and plumule at different day interval (cm)		
	2 days	4 days	6 days
Tissue:water	R:P*	R:P	R:P
1:5	-	-	-
1:10	-	-	-
1:20	0.2:0.0	0.5:0.1	0.8:0.2
1:40	0.4:0.0	0.9:0.2	1.4:0.3
Control	0.8:0.2	1.7:0.4	2.8:1.2

*(R:P = Radicle:Plumule)

favouring the allelochemicals containing plant to be forward in competition and dominate over colony.

Deviated reproductive feature

One common and unique characteristic of the Asteraceae family members is the presence of

pappus (persistent modified calyx). After maturation of the flower it helps the cypselum (fruit type of Asteraceae) to migrate over places and maintain dispersal. In *A. adenophora*, the pappus was observed to dissociate from the cypselum when it matures (Figure 3). In this way, the mature cypselum with deciduous calyx cannot disperse by



Figure 5: Pictorial evidences of pseudo-viviparous germination in *Ageratina adenophora* (a-l). Cypselas germinates when it still attached in capitulum, a clump of seedling fallen on lower substratum

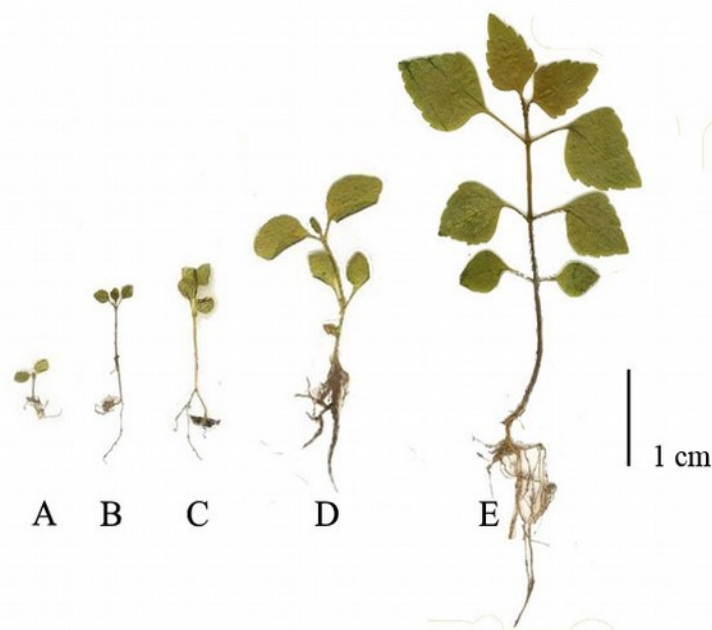


Figure 6: Different stages of seedling development (A-E) of *Ageratina adenophora*

parachute mechanism due to lack of effective pappus. This could be attributed to the fact that the plant's tendency to deviate the usual mode of dispersal of its close relatives and a very first step towards formation of dense colony surrounding its own habitat sectors.

Induced pseudo-viviparous germination and single colony formation

Aggressiveness of the species had also been reflected in its single generation individuals with vigorous growth rate in each colony (Figure 4) at a particular sector of its occurrence. Individuals of each colony mature and flower simultaneously. The clusters of flowers remain attached on the mother plants for significantly long span of time during the period of heavy monsoon. Heavy rain keep the head (capitulum) wet for long time that also prevent cypsela dispersal; on the other hand, cypsela whenever dispersed, cannot spread wide as heavy rain settle them on the ground. Very frequently, the cypsela had been found to get germinated with two minute cotyledons on the receptacle of head, still attached with the mother plant during monsoon (Figure 5). Subsequently, moist heads with many minute green propagules (each with two green protruded paracotyledons) become heavy and drop down entirely on the lower substratum and start to grow with normal series of seedling development (Figure 6). It was the beginning of the colony formation. As mentioned earlier, the incident of protruding cotyledonary body from the inflorescence still attached with the mother plant and emergence of radical only after shedding is called pseudo-vivipary. It was also observed that contemporary bunches of propagules from one or more adjacent plants contribute to same colony.

The event of vivipary, semi-vivipary or pseudo-vivipary is a regular feature of mangrove plants and also reported from some sea grass (Elmqvist and Cox, 1996), white clover (Majumdar *et al.*, 2004), chayote (Aung *et al.*, 1990), and some members of Cyperaceae (Gordon-Gray *et al.*, 2009) and Poaceae (Kuzmanović *et al.*, 2012; Milton *et al.*, 2008; Vega and Rugolo-de-Agrasar, 2006). In Cyperaceae pseudo-vivipary occurs by the development of plantlets adjacent to inflorescence or a floret within a spikelet (Gordon-Gray *et al.*, 2009). However, it is undoubtedly a striking character in a non-mangrove dicotyledonous Asteraceae member. In *Pennisetum setaceum* (Fountain grass) pseudo-vivipary is induced by inundation of immature inflorescences when its habitat (Gamka River in the Karoo National Park, South Africa) flooded (Milton *et al.*, 2008). Subsequently, plantlet production facilitates the spread of this species in seasonally flooding rivers in arid regions. Similarly, here *Ageratina* also gets sufficient moisture to keep the head

wet even after fruit maturation driving it towards the pseudo-vivipary. It supports the plants adaptation to higher reproduction rate as well as getting advantage in preventing the huge propagule wastage through fast flowing rainwater of hill slopes till the monsoon rain subside. Hence, the induced pseudo-vivipary in *A. adenophora* of Asteraceae is an adaptive advance trait for greater possibilities of survival of the species and aggressive colony formation.

Conclusion

Ageratina adenophora, a non-mangrove member from the Asteraceae family, shows a nice pattern of induced pseudo-viviparous germination, as a result of the high monsoon rainfall. By this mode of reproductive strategy clumps of germinating propagules develop on the fully matured heads still attached with the mother plants. Several clumps of such propagules falling on the lower substratum give rise to single generation colonies of more or less same size of individuals. The vigorous growth of the species removes other associated plants from its colonies and makes a dense cover on hill slope reducing the rate of soil erosion as well as chances of land slide.

Author contributions

All authors have contributed significantly. NCK has observed and documented the feature from natural condition. Both authors have performed the laboratory works and prepared the manuscript.

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