**Original Article****Effect of mechanical scarification on seed germination of selected weeds occurring in rice field**S. P. Paudyal<sup>1</sup>, S. Limbu<sup>1</sup>, B. D. Das<sup>2</sup>, N. Paudel<sup>3, 4\*</sup>, M. M. Rahman<sup>5\*</sup><sup>1</sup>Department of Botany, Trichandra Multiple Campus (Tribhuvan University), Kathmandu, Nepal.<sup>2</sup>Department of Botany, Mahendra Morang Aadarsh Multiple Campus, Biratnagar (Tribhuvan University), Nepal<sup>3</sup>National Institute of Horticultural and Herbal Science, Rural Development Administration, Wanju, 55365, Republic of Korea.<sup>4</sup>Department of Applied Plant Science, Kangwon National University, Chuncheon 24341, Republic of Korea.<sup>5</sup>Faculty of Biological Science, Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia-7003, Bangladesh**ABSTRACT**

Worldwide crop production is affected by different kinds of weeds that reduce yield in agricultural practices. Therefore, for the observation of weed seeds germination and the effect of mechanical scarification of the seed coats on the germination of selected weeds was assessed in the laboratory conditions. For the assessment of scarification effects on seed germination, the seven different kinds of weed genotypes were collected from rice fields in Kumaltar, Lalitpur, Nepal. The collected seeds were kept for one month and after de-husking of each species of 25 seeds was allowed to germinate in the laboratory with unscarred (normal) and scarred conditions on blotters at temperature  $25\pm 5^{\circ}\text{C}$  and experimental observation was done for 14 days. Among all the species, the highest germination (96%) was observed in scarred seeds but the lower germination (58%) was observed in normal seeds of *B. pilosa* compare to scarred seeds. The unscarred seeds of *A. conyzoides* seeds did not germinate at all, while the scarred seeds germinated up to 20%. But the seeds of *A. viridis* were germinated well in both scarred (84%) and unscarred conditions (80%). The germination of *E. colona* was also greatly influenced by scarification which increased from 4 to 32%. Similarly, the germination of *E. crus-galli*, *P. barbartum*, and *S. acmella* was also increased by 12, 20, and 4% respectively on scarification. It was observed that scarification of the seeds of the weeds under study quite satisfactorily improved the germination rate. The linear growth of the seedlings was also affected by the scarification of the seeds. In conclusion, the weed seeds management is needed for better production in the cropping system.

**Article History**

Received: 24 May 2021

Revised: 16 June 2021

Accepted: 19 June 2021

Published online: 30 June 2021

**\*Co-corresponding Authors**N. Paudel, M. M. Rahman, E-mail:  
nirojirauna@gmail.com;  
shilon83@gmail.com**Keywords**

Germination percentage, Linear growth, Productivity, Rice-weed, Scarred and unscarred.

**Introduction**

Weed biology is an integrated science with the target of minimizing the negative effects, as well as using and developing the positive effects of weeds (Badhaai *et al.*, 2021). The damage of global crop losses caused by the nuisance weeds about 76% compare to insect pests or pathogens (Oerke, 2006; Fried *et al.* 2017); yield losses to non-native weeds could amount to about 42% of crop production (Vilà *et al.*, 2004). Rice yields were being reduced by 23% from weeds growing above the rice canopy and by 21% from weeds growing below the rice canopy

(Gianessi, 2014). The ability to predict seed germination and emergence are essential to find better timing of mechanical, biological, and other management strategies.

Nepal has remained an agricultural country for a very long time. Almost 70% of people in Nepal still rely upon agriculture (NEP, 2016). Being an agricultural country, Nepal is undoubtedly a home for many crop weeds as well and the study of weeds especially their physiology, can't simply be left out as they play a vital role in affecting crop growth and productivity in many aspects. Among all the crops, rice dominates the cropping sequence in Nepal in

terms of land usage and production. It is cultivated in 1,439,525 ha of land making it the most cultivated staple crop in Nepal (CBS, 2015). Rice is a major food worldwide as well as in Nepal.

Numerous studies have been made on the weed species in the rice fields of Nepal from a floristic and ecological point of view (Joshi and Gretzmacher, 1998). Most of the studies reveal the presence of *Echinochloa crus-galli*, *Echinochloa colona*, *Fimbristylis spp.*, *Ageratum spp.*, *Cyperus rotundus*, *Cynodon dactylon* and *Ischaemum rugosum* as major weeds in the paddy fields (Bhatt 2006). Kennedy et al., (1980) stated that *E. crus-galli* is a serious weed in the flooded rice fields due to its ability to germinate even in the deep water. Dyer (1995) reported that changes in environmental factors such as light penetration, soil water content, soil fertility and temperature may indirectly lead to the alterations in phytohormone concentrations during the seed development, which can subsequently affect the dormancy status of the matured seeds. The seed coat is one of the main determinants of seed germination, vigor and longevity (De Souza and Filho 2001). A hard, impermeable seed coat may help to keep the embryo safe but at the same time, it hinders the interaction of the embryo with the various external environmental conditions required for a seed to germinate and delays the germination or can keep the seed dormant. Mechanical breaking, chemical scarring or manual removal of the seed covering almost always improves germination (Crocker, 1906, 1948; Crocker and Barton, 1953, Kimura and Islam, 2012). Furthermore, Paudel and Shrestha (2018b) stated that phenological changes are occurred in the case of herbs like *Mimosa pudica* and *Desmodium triflorum*. The productivity comparison to weedy check treatment due to the increase of critical growth and yield parameters of respective crops (Ghosh et al., 2020). Here is easy to the loss food supply in the rice system to reduce the physical and economic estimated loss (Ardakani and Simen, 2020).

The process of weakening or rupturing of the seed coats by mechanical or other means is called the scarification of seeds. The removal or scarring of the seed coats in most of the plants acts beneficial in promoting the germination rate but for some species, it may not be as beneficial or practical. The scarification of the seed coats can be done both mechanically and chemically. Mechanically, the seed coats can be weakened or scarred either by thrashing in the machines, rubbing in the rough surfaces (like sandpapers) or manual removal of seed coats without injuring the embryo. While chemically, the seed coat can be scarred by soaking in various mineral acids and bases of certain concentration for a particular time interval based on the nature of the seed coats. Effects of weeds on crops might also depend on weed origin (native versus non-native). Non-118 native plants have left behind natural enemies that keep their populations in check in their native 119 ranges (Maron and Vilà, 2001). Continuous cropping systems there is a steady reduction of organic carbon whereas under pasture a higher equilibrium content of soil carbon is maintained which is in balance with gains from net primary yield and losses from decomposition (Paudel, 2018a). The aim of the study was the effect of the unscarred and scarred conditions of weed seeds on the germination rates and correlate with the life sustainability in ecological conditions.

## Materials and Methods

### Study Area

Since, the study work has been done on the seeds of weeds of rice plants; the foremost material for the study is the seeds of the weeds. The weeds were collected from the rice fields in Khumaltar, Lalitpur, Nepal. Only the matured seeds were collected, dried, dehusked, and stored in paper bags to ensure proper aeration. The detail sample information is provided in Table 1.

**Table 1. Information on the collected seven seed genotypes of weeds from rice field of Kumaltar, Lalitpur, Nepal.**

S.N	Local name	Common name	Botanical name	Family
1.	Ganamane ghas or Gandhe jhar	Billygoat-weed or chick weed	<i>Ageratum conyzoides</i> L.	Asteraceae
2.	Lunde ghas	Slender amaranth or green amaranth	<i>Amaranthus viridis</i> L.	Amaranthaceae
3.	Tikhe kuro	Blackjack or beggar's stick	<i>Bidens pilosa</i> L.	Asteraceae
5.	Sama	Jungle rice or awnless barnyard grass	<i>Echinochloa colona</i> (L.)	Poaceae
4.	Sama ghas	Barnyard grass	<i>Echinochloa crus-galli</i> (L.)	Poaceae
6.	Pire bikh	Knotweed or smartweed	<i>Polygonum barbatum</i> L.	Polygonaceae
7.	Lattu ghas	Toothache plant	<i>Spilanthes acmella</i> (L.)	Asteraceae

### Experimental Set up

The experiment was conducted in the laboratory of Tri-Chandra Multiple College, Department of Botany about a month after the collection and storage of the seeds. Two experiments were conducted in this study on the weed seeds which mainly concerns with germinating the seeds under the controlled laboratory condition by using the Blotter method. The first experiment focused on germinating the seeds without scarring them. And, the second experiment focused on germinating the seeds after scarring their seed coats. The seeds for the experiment from the stored lot were selected using the simple gravity separation technique where the seeds were washed with distilled water and the ones floating were assumed unhealthy and discarded. For the first experiment, some fine seeds were selected inspecting them by using a magnifying lens. The seeds were then soaked in distilled water overnight. The simple mechanical scarification technique was applied for seed scarification

either using blades or sandpapers. The seeds of the weeds *Ageratum conyzoides*, *Bidens pilosa* and *Spilanthes acmella* were carefully scarred using a new sharp blade at their tips only, observing under the magnifying lens. Since the seeds of *Amaranthus viridis*, *Echinochloa crus-galli*, *Echinochloa colona* & *Polygonum barbatum* were tiny and smooth, these seeds were scarred rubbing in the coarse sand paper. The sufficient seeds were scarred, selected and soaked in distilled water for 24 hours before germination testing.

### Blotter preparation, seeds placement, and incubation

The weed seeds were to be germinated under laboratory-controlled condition. For this purpose, the blotter method was selected as it was more convenient and easily accessible. Three blotter papers were placed on each petri-dishes and completely soaked in excess distilled water. Finally, 25 seeds were placed on the wet blotter papers in the two concentric rings with one seed in the centre using sterile forceps

maintaining a sufficient gap between the seeds. The petri-dishes with the seeds were properly labelled and incubated at  $25 \pm 5^{\circ}\text{C}$  for germination. The distilled water was added every 48 hours in the petri-dishes to ensure that the blotters are moist and the seeds are getting sufficient moisture for imbibition to promote germination throughout the observation duration

### Data collection

The regular observation was made for 14 days between the dates December 12 to December 26. The seeds were checked for any sign of germination using a hand lens for convenience. Numbers of germinated seeds were recorded on daily basis. The linear lengths of the seedlings were measured on the last day of the observation using a glass slide on graph paper. Germination percentage is an estimate of the viability of a population of seeds. The germination% of the all species of weeds in both unscarred and scarred condition was calculated at the end by using:

$$\text{Germination\%} = \frac{\text{No of seeds germinated}}{\text{Total no. of seeds sowed}} \times 100\%$$

### Results and Discussion

In the present study, the germination of *A. conyzoides* seeds showed significant progress on scarification. The germination (%) of the scarred *A. conyzoides* was 20% while the unscarred seeds showed 0% germination (Figure 1). Sauerborn and Koch (1988) have said that the optimum temperature for the germination of the *A. conyzoides* seeds ranges from 20-25°C. The *Ageratum* seeds germinate about 50% after shading, and the seeds are positively photoblastic, and rest of the 50% seeds tend to remain dormant (Sauerborn and Koch, 1998).

*A. viridis* seeds germinated well, with or without scarring. The fresh seeds of the *Amaranthus* spp. did not germinate easily as they need an after ripening duration. Barton (1945) stated that the seeds of *Amaranthus* and *Rumex* spp. are dormant when freshly harvested and they will only germinate at specific temperatures, but during month's dry storage the sensitivity alters allowing them to germinate over a wide range of temperature. Since, the seeds used for the study were a month old so, the seeds germinated as soon as they were sown. The seeds of *A. viridis* started germinating on the 2<sup>nd</sup> day of the incubation in both scarred and unscarred cases. The germination% for *A. viridis* was high in both experiments, 80% and 84% for unscarred and scarred seeds respectively. Thomas et al., (2006), found out that the optimum constant temperature for the germination of the *A. viridis* is 30°C. It was also observed in the previous study, the seed germinate better in the acidic than with basic pH conditions (Thomas et al., 2006). Chauhan and Johnson (2009a) stated that the germination of both *A. spinosus* and *A. viridis* was stimulated by 35/25 and 30/20°C fluctuating temperatures and light.

The germination of the *B. pilosa* seeds was highly effected by the mechanical scarring of the seeds. At the end of the experiment, altogether 14 out of 25 unscarred and 24 out of 25 scarred seeds germinated. The scarification of the *Bidens* seeds increased the germination by 40%. The scarred *B. pilosa* seeds were the ones having the highest germination in this study with germination of 96%. Forsyth and Brown (1981) had tested the germination of the short and long seeds of the *B. pilosa* under different conditions. The germination of the seeds on scarification was 92-100% from 20% for the short *Bidens* seeds and 100% (which was the same in unscarred condition too) for the longer seeds. The percentage

germination was increased along with the germination rate. The similar pattern can be observed in the present study. However, the seeds taken here is mixture of both long and short seeds. Reddy and Singh (1992) observed that the germination of *B. pilosa* was favored by both 12 hr photoperiod and 24 hour dark regime. The optimum temperature ranges from 25/20 to 35/30°C + (day/night, 12/12 hr).

Only one seed of *E. colona* was able to germinate when not scarred on the 13<sup>th</sup> day of the incubation while the scarred seeds successfully started germinating from the very 1<sup>st</sup> day after incubation and eight seeds germinated till the last day of the observation. Scarification of *E. colona* seeds brought significant improvement in germination. Chun et al., (1987) have reported that *E. colona* seeds did not require a period of after ripening for breaking dormancy and removal of the seed coats increased the germination in dark. Chauhan and Johnson (2009b) suggests that the seeds of *E. colona* are positively photoblastic and require light for its germination. The tested temperatures (35/25, 30/20 and 25/15° C) in laboratory didn't influence the germination.

The weeds tend to have the enormous ability to rapid population establishments once they get the favorable environment with different kinds of dormancy and germination periods. The species that have been studied here have no doubt that they are weedy in nature. The seven weeds that have been studied here are recognized as weeds in the rice fields by many other workers before. Manandhar (2004) have mentioned *Echinochloa colona*, *Echinochloa crus-galli*, *Ageratum conyzoides*, *Spilanthes acmella*, *Bidens pilosa*, *P. barbatum* along with many other weeds to be present in the rice field of Kirtipur area. Similarly these weeds and *A. viridis* have been mentioned from the paddy fields of Mahendranagar too by Bhatt et al., (2009). Rajbhandari and Joshi (1998) have also recorded around 364 crop weeds from various different parts of Nepal. All the weeds taken in this study have been mentioned as the crop weeds of Nepal.

Only one seed of *E. colona* was able to germinate when not scarred on the 13<sup>th</sup> day of the incubation while the scarred seeds successfully started germinating from the very 1<sup>st</sup> day after incubation and eight seeds germinated till the last day of the observation. Scarification of *E. colona* seeds brought significant improvement in germination. Chun et al., (1987) have reported that *E. colona* seeds did not require a period of after ripening for breaking dormancy and removal of the seed coats increased the germination in dark. Chauhan and Johnson (2009b) suggests that the seeds of *E. colona* are positively photoblastic and require light for its germination. The tested temperatures (35/25, 30/20 and 25/15° C) in laboratory didn't influence the germination.

Unlike *Echinochloa colona*, *E. crus-galli* significantly germinated in both unscarred and soaked and scarred and soaked state. The unscarred seeds (44%) successfully germinated while the number of scarred seeds germinated on the last day reached 14 (56%) (Figure 1). Shi-Jean et al., (1987) also found out that the germination in the *E. crus-galli* can be induced by the wounding the seed coat up to 10-70% on the basis of the location of the scar or wound. More germination was observed when the coat covering the radical was removed. Yamasue et al., (1992) also supported that the percent germination of *E. crus-galli* increased by its seed scarification and treatment with sulphuric acid and sodium hydroxide. They achieved 95% germination by soaking the seeds of *E. crus-galli* in conc. sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) for 15



min. The germination of the *P. barbatum* subsequently increased on the scarification of the seeds. It was also observed that the scarred seeds initiated the germination quicker than the unscarred ones. The increase in the germination was 20% when the seeds of the *P. barbatum* were scarred. Colpetzer and Goldstein, (2003), observed from his experiments, in the achenes of *Polygonum perfoliatum*, both coat imposed and chemical imposed mechanism enforce its dormancy. Coat- imposed dormancy was overcome by scarifying the seeds for 60 mins in 93% sulphuric acid while the chemical imposed dormancy was overcome by storing the freshly harvested seeds for at least one week at 23°C and stratifying the seeds in de ionized water at 4°C for four hours. Similar case was observed by Courtney (1968) in *Polygonum aviculare* species whose germination was stimulated by exposing the seeds to period of low 4°C and high (23-25°C). Seeds of *Porophyllum ruderale* are sensitive to brightness, the absence of light drastic reduction of the germination of seed that means light influenced (Yamashita et al., 2008).

The scarring of the seeds of the *S. acmella* had no significant change in the germination of the seeds. The germination pattern was almost similar when the normal seeds and scarred seeds of this species were grown. The seeds didn't germinate until the 6<sup>th</sup> day of the incubation of the normal seeds while the first scarred seeds germinated on the 5<sup>th</sup> day of the incubation. 10 unscarred and 11 scarred seeds germinated until the last day of the observation. The increase in the germination of *S. acmella* was 4% by the scarification. *S. acmella* is widely used as medicinal plant in ethnopharmacology (Paulraj et al., 2013). Seeds scarified with concentrated sulphuric acid for 10 minute is better germination without scarification (Paudel and Rai, 2018c). The matured seeds of *S. acmella* have been found to germinate within a week usually by many cultivators. The fresh seeds possess the dormancy and require certain after ripening period which can be overcome by the dry storage. Effect of sowing methods and weed control treatments and their interactions on weeds, yield and yield components of wheat Giza 168 cultivar grown under new reclaimed land conditions (Hassanein et al., 2020). Weed infestation causes reduce the productivity in all cultivars even in partially weedy condition direct seed rice systems indicating the importance of weed management (Ahmed et al., 2021).

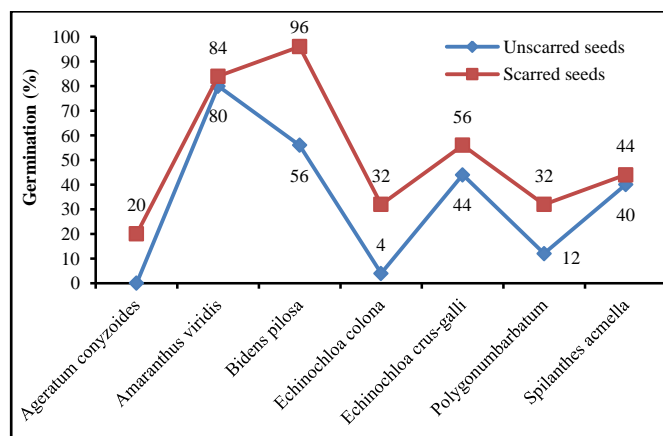


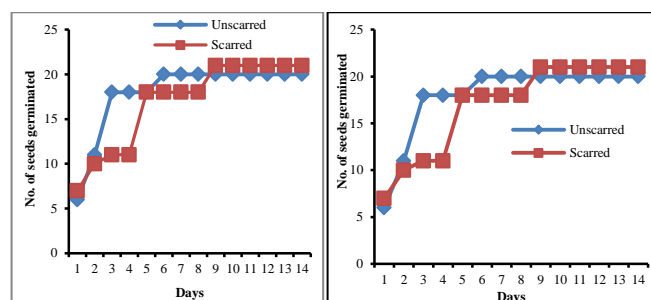
Figure 1. Germination effect on weed seeds in scarred and unscarred condition.

### Linear growth

The linear seedling growth of the weeds was also affected by the scarification. In the species *A. viridis*, *E. colona* and *P.*

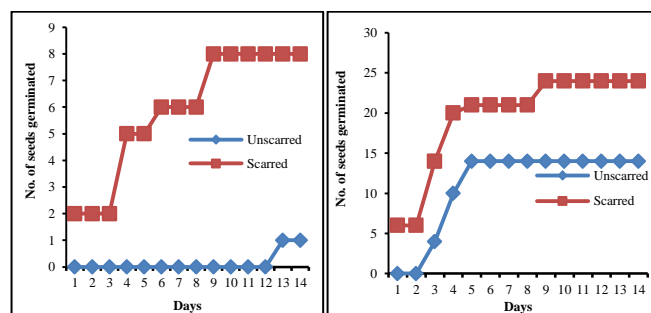
*barbatum* the linear growth increased by scarification of the seed coats while in case of *B. pilosa*, *E. crus-galli*, and *S. acmella* did not show the linear growth of the seedlings (Figure 2). Acidic condition is useful for the germination. *B. pilosa* seeds was highly effected due to the mechanical scarring of the seeds for that the percentage germination was increased along with the germination rate. Among the seven genotypes of weed seeds, the *E. colona* seed showed a unique feature which is able to germinate without scarred on the 13th day of the incubation while the scarred seeds successfully started germinating from the very 1st day after incubation and eight seeds germinated till the last day of the observation. In case of the *P. barbatum*. *S. acmella* had no significant change in the germination of the seeds (Figure 2). In case here the germination flow almost similar when the normal seeds and scarred seeds of the weeds grown the linear seedling growth is also affected by the scarification. The germination pattern was almost similar when the normal seeds and scarred seeds of these species were grown. The linear seedling growth of the weeds was also affected by the scarification (Figure 2).

The major threat to rice productivity and quality due to the culture practice, biological as well as physiological practice that the sustainable weed management control of the crop production (Paiman et al., 2020). Integrated novel approach idea will assist farmers in coping with the challenges of the weed management for sustainable rice production (Darmola et al., 2020). Nikolic et al, (2021) noted that the crop residues can be viable option for the weed control would be great utility for farmer's decision making agriculture and conventional agriculture. The weeds *Desmodium triflorum* changes the benefits of physio-chemical properties in soil. The management and valuable of changes in physio-chemical in soil due to the *Desmodium triflorum* (Paudel et al., 2017).



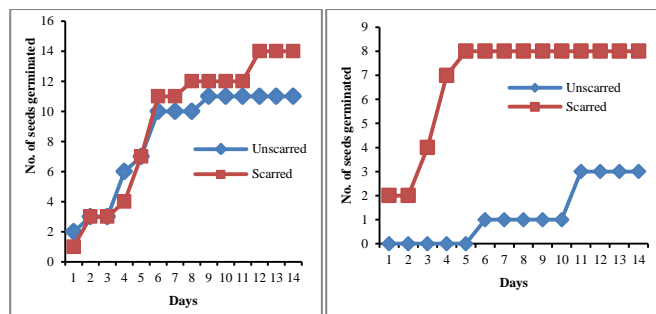
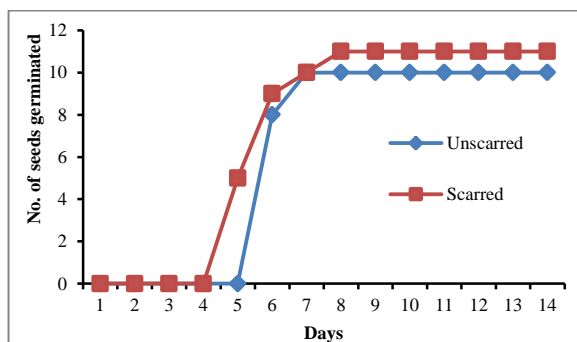
A. *Ageratum conyzoides*

B. *Amaranthus viridis*



C. *Bidens pilosa*

D. *Echinochloa colona*

E. *Echinochloa crusgalli*F. *Polygonum barbatu*G. *Spilanthus acmella*

**Figure 2.** Germination effects of seven different genotypes such as **A.** *Ageratum conyzoides*; **B.** *Amaranthus viridis*; **C.** *Bidens pilos*; **D.** *Echinochloa colona*; **E.** *Echinochloa crusgalli*; **F.** *Polygonum barbatu*; **G.** *Spilanthus acmella* species of weed seeds with scarred and unscarred condition.

The rapidly increasing world population is problematic for hungry so it is urgent need to maintain the quality of rice; weeds problem must be sorted out on priority basis. Ozaslan (2015) state that the weed scientists must determine the important weeds for different crops particularly rice in the above addressed region and devise effective management strategies scientists, engineers, economists, sociologists, educators, farmers, land managers, industry personnel, policy makers, and others willing to focus on weeds within whole farming systems and land management units should be develop a new approach (Limman *et al.*, 2016). Photocontrol of weed germination via photochromes is suited to reduce weedless in agricultural field by means of without light (Hatmann, 2016).

Besides this, mefenacet, fentrazamide and cafenstrole had excellent controlling effects on the Acetyl-CoA Carboxy lase (ACCase) and ALS inhibitors resistant when they were applied within the two leaf stage (Park *et al.*, 2011). The activity methanogens and diversity which factors controlling the ecology and possible interaction for the source of relationship of the greenhouse gas emission (Alpana *et al.*, 2017). Paudel and Kang (2018d) observed that algal is biofertilizer for *coffee arabica* which can reduce the harmful for soil. Furthermore, the plant residue increase the physiochemical properties of soil (Paudel *et al.*, 2021)

## Conclusion

Weeds are one of the most limiting factor on rice cultivating system depending upon the weed density, period of their infestation. To control the weeds, farmers mainly depends upon the chemical herbicides. However, it causes the resistance to overuse of herbicide in many weed species so that gives the negative effect. So, it is concluded that there is urgent needed for ecologically stable process. Needs to crop rotation and hand practice methods is reliable for the weed

control and as well as prevent the excessive herbicide used for environment control. In this study, the effect of seed germination rates with respect to the unscarred germination of seven different genotypes of seeds. Among them, *A. conyzoides* seeds significant progress due to scarification. *B. pilosa* seeds was highly effected due to the mechanical scarring of the seeds for that the percentage germination was increased along with the germination rate. Among the seven genotypes of weed seeds, the *E. colona* seed showed a unique feature which is able to germinate without scarred on the 13th day of the incubation while the scarred seeds successfully started germinating from the very 1st day after incubation and eight seeds germinated till the last day of the observation. But there was a little or no changes in the germination of the seeds of *P. barbatum* and *S. acmella*. The germination pattern was almost similar when the normal seeds and scarred seeds were grown. The linear seedling growth of the weeds was also affected by the scarification. This research would be used for better management of weed seeds of paddy field.

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