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Effects of leaves extract of *Eichhornia crassipes* on seed germination and seedling growth of *Pinus roxburghii* and *Bauhinia purpurea*

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ABSTRACT

E. crassipes is threatening the aquatic diversity in famous lakes of Nepal. Thus, this study was objectively carried out to evaluate the effects of extract of E. crassipes on seed germination and seedling growth of Pinus roxburghii and Bauhinia purpurea, assess the soil nutrients according to proportion of extract and distilled water. Leaves of E. crassipes were collected from Phewa lake. Next, laboratory techniques were applied for oven drying, soaking, shaking incubator, centrifuging, filtering and dilution. Altogether 120 polypots, 60 each from Pinus roxburghii and Bauhinia purpurea were used for seed germination. So, 15 replicates for each block representing concentration of extract: distilled water (75:25, 50:50, 25:75 and control). Records were taken between 18th March and 24th April, 2018. Temperature and humidity were recorded on 0700, 1300 and 1900 hours. Soil N, P, K, C and pH of different concentrations were analyzed in the lab. It was found that the highest germination in 25:75 concentration specifically 14 and 11 in P. roxburghii and B. purpurea respectively. The diameter of P. roxburghii was the highest in 25:75 with 1.52 mm while it was the highest in 75:25 with 1.34 mm of B. purpurea. The average height of seedling growth of P. roxburghii was the highest in 25:75 concentration having 8.24 cm. The highest record of needles 12 was found in 75:25 concentration. The repeated measures ANOVA showed that there were significant differences in seed germination and seedling growth at 95% confidence level. The nutrient analysis showed that, the highest N%, P_2O_5 kg/ha, K_2O kg/ha and C% were 0.22, 30.30,1359.60 and 2.56 respectively in P. roxburghii at 75:25 concentration. The R^2 values of temperature vs germination was 0.16 and this was 0.01 for P. roxburghii and B. purpurea respectively. This research will be useful tool to utilize this for efficient organic fertilizer for forest nursery work.

Keywords: *E. crassipes, P. roxburghii, B. purpurea, extract, germination, soil nutrient*

INTRODUCTION

Eichhornia crassipes is a floating "obligate" (requiring a wet habitat) plant belonging to the family Pontederiaceae. *Eichhornia crassipes* which is considered to be originated from the Amazon and has disseminated very quickly in various tropical and sub- tropical countries of Latin America and the Caribbean, Africa, Southeast Asia and the Pacific (Wolverton & Mc Donald, 1979, Charudattan *et al.*,1995). Water hyacinth is an allelopathic aquatic plant (Gross, 2010) which has gained notoriety as an invasive weed worldwide (Chai *et al* ., 2013). This capacity of rapid growth and propagation is the major problem for management of *E. crassipes* in aquatic ecosystem. In addition, this weed is also listed on IUCN's 100 most dangerous invasive species (*Tellez et al.*,, 2008) and included on world's top 10 worst weeds (*Aboul-Enein et al.*, , 2011).

E. crassipes is commonly thought to have entered Nepal from India due to the geographic proximity of the two countries. It was first reported in Nepal in 1966 as cited by (Maharjan & Ming, 2012). *E. crassipes* has been infested in several famous lakes particularly Phewa, Bishazari, Begnas etc in Nepal. Phewa Lake has been one of the most important touristic assets of Pokhara, hampered badly due to the invasion of this weed. The beauty of the lakes is affected due to infestation and the consequences are effect on ecotourism business, irrigation system and suffocation to the aquatic organisms. Huge amount of money has been invested to clean *E. crassipes* from the lakes. Therefore, the proper management option is needed to control the weed. In fact, the proper utilization of the weed will be better option to control the weed. However,

study regarding the effect of leaves extract on seed germination and seedling growth of common timber and fodder species like *Pinus roxburghii* and *Bauhinia purpurea* respectively has not so far done in Nepal. Leaves extract of *E. crassipes* can be utilized as a organic manure which will reduce cost of chemical fertilizer (Chai, 2013). The seed germination and seedling growth depends upon the soil fertility (UNEP, 2006, Vidya & Girish, 2014) but the research regarding the effect of leaves extracts on soil nutrient has not done so far in Nepal. Therefore, this study was objectively carried out to assess the effects of leaves extracts of *E. crassipes* on seed germination and seedling growth of *Pinus roxburghii* and *Bauhinia purpurea.* In addition, the evaluation the effect of leaves extract on soil nutrient (N, P, K, C) and pH based is another aim of this research.

Materials And Methods

E. crassipes leaves were collected from Phewa lake from Pokhara, Nepal. Pokhara is a small valley lying in central Nepal at latitude and longitude 28.2380° N, 83.9956° E respectively. Valley has an average temperature of 20.6 °C and rainfall averages 3474 mm. Phewa Lake borders the valley towards south at an elevation of about 827 meters from asl. The seed of *P. roxburghii* and *B. purpurea* were collected from Tree Improvement and Silviculture Component, Nepal.

Laboratory work

E. crassipes leaves were cleaned and dry patted using clean cloth. The chemical from *E. crassipes* leaves was extracted (Chai *et al.*, 2013) at lab in Nepal Academy of Science and Technology. In fourth day of seed sowing, 4 drops of extract prepared was applied using a dropper. Extract was applied in intervals of 4 days for 4 times. Four drops of extract were applied for the fifth time after 23 days of sowing to check effects on seedling growth.

Nursery setup:

Altogether 120 polypots, 60 each for *Pinus roxburghii* and *Bauhinia purpurea* were used for seed germination. The seeds were sown on 18th March and recorded till 24th April, 2018 for germination and seedling growth. Nursery plot was established using randomized complete block design (RCBD) inside glass house having 15 replicates for each block. Each block represents the concentration of *E. crassipes* extract: distilled water (75:25, 50:50, 25:75 and control). All polypots except control pots were treated using different concentrations. Daily observation and recordings were noted for 34 days and 38 days for the seed germination and seedling growth of *Pinus roxburghii* and *Bauhinia purpurea* respectively. Moreover, temperature and humidity were recorded for three timings (0700, 1300 and 1900 hours).

Measurement and recoding:

Germination percentage of *P. roxburghii* and *B. purpurea* under distinct concentrations of leaves extract of *E. crassipes* were recorded. Similarly, after the germination of seeds, seedling height, base diameter was measured and no. of leaves were counted on a one-day interval basis and recorded.

Soil analysis:

Soil N, P, K, C and pH of different concentrations were analyzed using Kjedahl method, Bray's method no.1 for acid soils (Bray and Kurtz, 1945), Flame photometric method (Toth and Prince, 1949), Walkley and Black (1958) Volumetric method and pH meter (DoAC, 2011) respectively.

Statistical analysis:

The collected data were analyzed using the descriptive analysis and inferential analysis. The normality of the data was tested using Kolmogorov and Shapiro-Wilk test. The One-Way ANOVA, Tukey's b, Repeated Measures ANOVA and correlation (temperature vs germination and humidity vs germination) were used as inferential statistics for the data which showed normal and Kruskal-Wallis for not normal (Kothari, 2004).

Results

Results illustrates the effect of extract on seed germination and seedling growth of *Pinus roxburghii* and *Bauhinia purpurea.* The results also show perception of people on *E. crassipes* in Phewa lake. This result also determines the effect of extract on soil nutrient and pH.

Effects of extract of *E. crassipes* on seed germination of *Pinus roxburghii* and *Bauhinia purpurea*

Total 10 recordings were taken for *Pinus roxburghii* on everyday basis regularly after 10th Day of seed sowing i.e. 28th March, 2018 to 6th April, 2018. Early germination is seen in 75:25 concentration and 50:50 concentration. However, it shows that germination was in peak from 31st March to 3rd April with 4 germinations in 25:75 concentration in both days but it was the lowest germination was observed in control concentration (figure 1).

The one way ANOVA showed that there was no significant effect on the germination percentage of *P. roxburghii*.

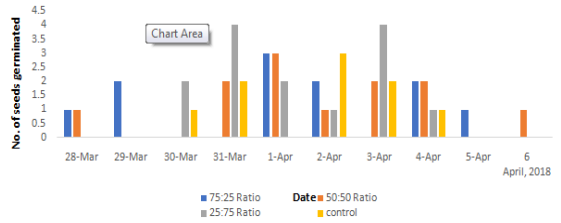

Fig1. *Germination recording in P. roxburghii*

In the case of *B. purpurea*, early germination was observed after 11th day of seed sowing in 50:50 and 25:75 concentration. Peak germination was shown in 18th April in 25:75 concentration with 3 seeds germination (fig. 2).

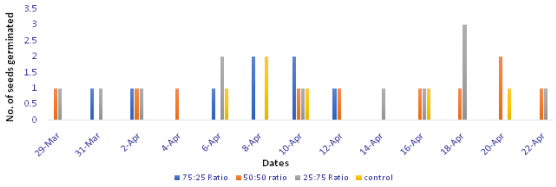

Fig2. *Germination recording in B. purpurea*

Effects of extract of *E. crassipes* on seedling growth of *Pinus roxburghii* and *Bauhinia purpurea*

Results show the effect of different concentration on seedling growth (changes in diameter, height and no. of leaves) in *Pinus roxburghii* and *Bauhinia purpurea*. The height and number of needles recording in the case of *P. roxburghii* was noted from 6th April to 20th April, 2018 while in the case of *B. purpurea* recordings were noted from 14th April to 25th April, 2018

Effect of extract on diameter growth of *P. roxburghii* and *B. purpurea*

In the initial recordings, diameter was seen the highest in 75:25 concentration with average diameter being 1.82 mm while average of diameter readings of 25:75 concentration was 1.69 mm, 50:50 concentration was 1.58 mm and 1.042 mm in the case of control as the least value (fig 3).

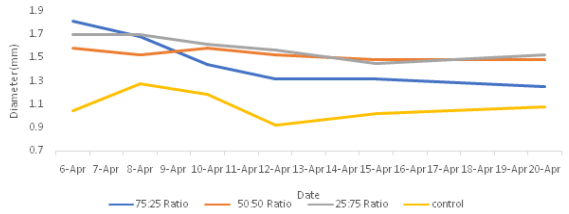

Fig3. *Effect of different concentration on diameter of P. roxburghii seedlings.*

In the case of *Bauhinia purpurea* diameter was seen highest in 50:50 concentration initially with 1 mm. There was not much difference in average diameter initially between 75:25 concentration with 0.94 mm and 50:50 concentration. In the end of the study 75:25 concentration resulted out to have the highest average diameter of 1.34 mm Average diameter of control was seen least from beginning of data recording till the end (Fig 4).

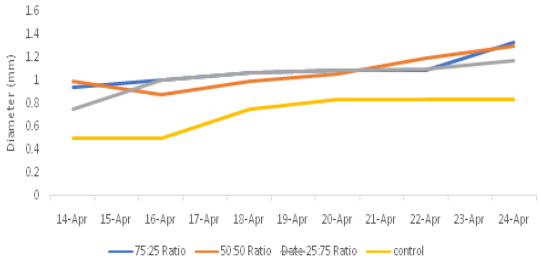

Fig4. *Effect of different concentration on diameter of B. purpurea seedlings.*

The Repeated measures ANOVA showed that there was significant effect of different concentration extract treatment on diameter growth at 95% confidence level since p-value is less than 0.05.

Effect of extract on height of *Pinus roxburghii* and *Bauhinia purpurea*

Height recordings were found much similar on the first recording. It was observed that average height was 6.25 cm in 75:25 concentration, 6.22 cm in 50:50 concentration, 6.50 cm in 25:75. At the end of the study on 20th April, it was found that average height of seedlings in 25:75 concentration was maximum with 8.24 cm but it was the least in control concentration with 7.31 cm (Fig 5).

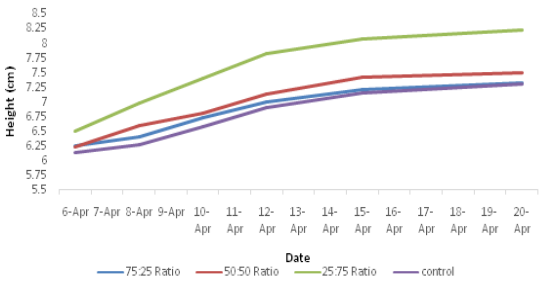

Fig5. *Effect of different concentration on height of P. roxburghii seedlings*

Initially average height of *B. purpurea* was almost equal in 50:50 concentration and 25:75 concentration with 3.7 and 3.5 cm respectively. At the end of study, seedlings in 50:50 concentration showed highest average with 5.81 cm but it was least in control concentration with 4.35 cm (Fig. 6).

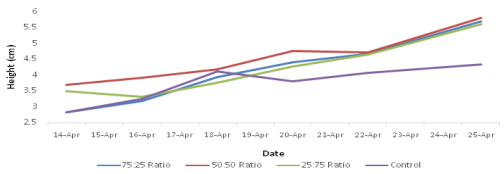

Fig6. *Effect of different concentration on height of B. purpurea seedlings*

Similarly, Repeated measures ANOVA showed that there was significant effect of different concentration extract treatment on height growth at 95% confidence level since p-value is less than 0.05.

Effect of extract on number of needles and leaves of *Pinus roxburghii* and *Bauhinia purpurea*

In the case of *P. roxburghii* average number of needles were constant in all concentration throughout the study. However, results show that average number of needles were highest in 75:25 concentration with 12 number of needles. 50:50 concentration and 25:75 concentration both had 11 while constant had the least number with 10 needles (Fig 7).

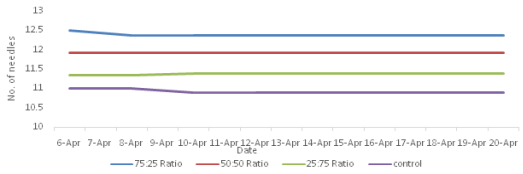

Fig7. *Effect of different concentration on number of needles in P. roxburghii seedlings*

In the recording, it was found that initially 50:50 concentration and control had equal as well as highest number of leaves. But towards the end of study, number of leaves were highest with 4 leaves in 50:50 concentration. However, the least number of leaves with 3 leaves was found in control (fig 8). Meanwhile the Repeated measures ANOVA showed that there was significant differences in number of leaves according the extract in the seedling of both species.

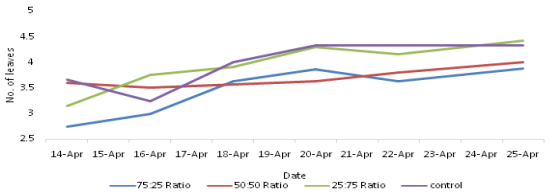

Fig8. *Effect of different concentration on number of leaves in B. purpurea seedlings*

Effect of different concentration on pH, N, P, K and C of soil

The nutrient in mycorrhizal soil was also affected due to extract. The nitrogen percentage was 0.18 in control whereas it was found to be 0.22 in 75:25 concentration. The pH of control was 6.53 while pH in 75:25 concentration was 6.4. The P_2O_5 was found to be increasing with the increase in concentration of extract. P_2O_5 was found to be 18.54 kg/ha in the case of control while it was 30.3 kg/ha in the case of 75:25. The K_2O was the highest 1359.60 kg/ha in 75:25 whereas it was the lowest 1206 kg/ha in control (Table 1). The highest OM % was found 0.31. in 75:25 while it was least only 0.25 in control.

The soil nutrients in *B. purpurea* was differed according to the proportion of extract. The pH was the highest 6.73 in control but it was least 6.70 in 75:25. In the case of soil used for germination of *B. purpurea*, soil test resulted that nitrogen percentage was found to be increasing with the increase in concentration. The N% was highest around 0.31 in 75:25 but it was the least only 0.25 in Control soil. The highest P_2O_5 was found to be 125.66 kg/ha in 50:50 concentration. increasing with the increase in concentration. The K_2O was 2839.8 kg/ha highest in 75:25 but it was the least 2613.00 kg/ha in Control soil. OM% was the highest 3.08 in 50:50 while it was the least only 2.96 in Control soil (Table 1).

Table1. *Soil category and nutrients*

Soil category	Concentration	N%	P_2O_5 kg/ha	K_2O kg/ha	C (OM%)	pH
P. roxburghii (Mycorrhizal soil)	75:25	0.22	30.3	1359.6	2.56	6.4
	50:50	0.2	27.08	1313.2	2.35	6.46
	25:75	0.19	22.66	1259.6	2.22	6.5
	Control	0.18	18.54	1206	2.15	6.53
Soil of *B. purpurea*	75:25	0.31	120.48	2839.8	3.61	6.7
	50:50	0.25	125.66	2733.6	3.08	6.75
	25:75	0.26	119.48	2639.8	3.05	6.75
	Control	0.25	98.82	2613	2.96	6.73

One-way ANOVA and Tukey's b showed that there was significant effect of different concentration on N, K_2O, pH, C, P_20_5 value of soil in *P. roxburghii* and *B. Purpurea*.

Temperature and humidity inside glasshouse

Temperature was low during the initial days with 11.4 °C on the second day of sowing however was highest on 4th April with 21 °C at 0700 hours. Day time temperature at 1300 hours was however least on 14th April with 23 °C inside the glass house whereas highest reached up to 46 °C on 1st April. Evening temperature measured at 1900 hours showed highest, 26 °C on 22nd April and least, 6 °C on 22nd March.

Overall average temperature nevertheless was in between 20 °C to 30 °C (fig 9).

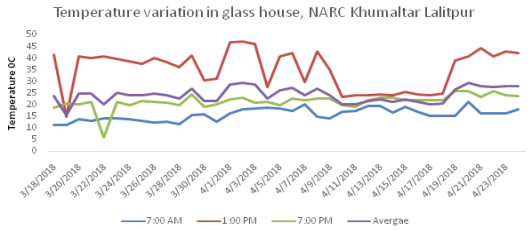

Fig9. *Temperature recording from 18th March (date of sowing) to 23rd April*

Humidity variation inside glasshouse and its correlation with germination

Humidity inside the glass house was least on the final day of recording with 49% while highest on 19th April with 91%. Humidity was found high during 0700 hours and low in 1300 hrs. At 1300 hours, humidity was 67% on 7th April whereas it was low with 10% on most of other days. At 1900 hours humidity was least with 32% and highest with 68% on 30th March and 4th April respectively. The average humidity however was in between 30% to 70% (fig. 10).

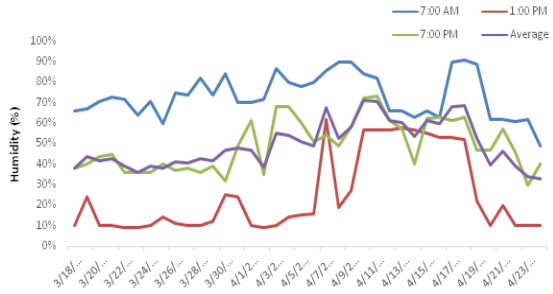

Fig10. *Humidity recording from 18th March(date of sowing) to 23rd April*

DISCUSSION

Seed harvesting was done on March following (Jackson,1994) which was little early harvesting than usual. This might be the reason behind overall germination and seedling growth delay in the case of *B. purpurea*. Germination started after 11 days of seed sowing and ended till 35 days which did not support (Jackson, 1994) who has advocated in his book that germination begins 5-10 days after sowing and is completed 1-2 weeks later for both *P. roxburghii* and *B. purpurea.*

Despite early germination observed in 75:25 concentration and 50:50 concentration in *P. roxburghii* the highest number of seeds germinated in 25:75 concentration. This may be due to fertilizer toxicity due to treatment 75:25 concentration where there was the highest concentration of extract (Hilderman, 2014). This result is in support with (K.C. & Mandal, 2017).

After the 2nd application of the extract after 4 days of sowing, too much fertilizer might have played toxic role in germination of seeds. This result is in oppose to results by (Lata, 2013) who experimented with water hyacinth manure on *Coriandrum sativum* and revealed positive response with increase in concentration of manure. Utilization of *E. crassipes* as a manure (Labrador, *et al.*, 2015) play a significant role in forest science specifically nursery techniques.

In the case of seed germination 6.50, 0.19, 22.66, 1259.60 and 2.22% are found to be appropriate pH, N, P, K and C (OM%) for *P. roxburghii* while 6.75, 0.26%, 119.48, 2639.80 and 3.05% are found appropriate pH, N, P, K and C (OM%) for *B. purpurea.*

In case of *P. roxburghii*, diameter of few seedlings was found to be decreasing negligibly with the increase in height. Seedling growth in control however had the least amount of germination and least average diameter, height and number of leaves. Extract showed better growth performance in all three aspects. These results are in support with both (Lata & Dubey, 2007) and (Vidya & Girish, 2014) who worked with water hyacinth manure and (K.C. & Mandal, 2017) who worked with the extract of *Mikania micrantha*. This enhanced growth might have been possible because the plants were able to absorb higher amount of nutrients from the soil where extract was applied.

Organic fertilizer from water hyacinth have very significant effect on the examined variables like plant height, number of leaves. Results are in support with (Talkah, 2015) whose findings showed that height growth and number of leaves in samples where water hyacinth manure had been used showed better performance than control. Findings of the research also shows positive relationship in terms of diameter and

height with the application of phosphorus. Similar results were obtained by several authors (Cuesta B, 2010) and (Razaq, *et al.*, 2017).

There was increase in N, P, K and OM% with the increase in concentration of *E. crassipes* extract. Similar results were found from several authors. Results obtained from the soil test show that soil had optimum amount to N, P, K for growth of other forest and agriculture crops which is in support to the findings of (Kafle *et al.*, 2009) who experimented with water hyacinth composting. This was also supported study done by (Aboul-Enein *et al.*, 2011) and (Aftab, 2017) who advocated that water hyacinth has good N, P, K absorbing capacity from water and thus can be used as a good source of compost material (Osoro, *et al.*, 2014). In addition, their study also highlighted that pH range above or below the range 6.0- 7.5. The pH value was found 6.4 and 6.7 in *P. roxburghii* and *B. purpurea* in 75:25 concentration respectively which is considered ideal for better plant growth. Plants are able to uptake nutrients only with optimum pH value in soil (Jackson,1994).

Both *P. roxburghii* and *B.purpurea* germinated in bulk with the average temperature of 25- 35 °C. Average temperature of Kathmandu valley, initially 19 °C started to increase after a week of sowing and reached till 25-30 °C later. This temperature was found to be directly proportional to seed germinability. Seed germination happened at bulk in the case of *P. roxburghii* however pattern was slow for *B. purpurea*. Temperature went on increasing and reached 45 °C inside glass house in 1300 hrs recording which might have caused obstacle for the germination of *B. purpurea*. Such study was done by (Ghidiyal, 2009) whose results showed temperature above 40 °C was inversely proportion in seed germination.

Conclusion And Recommendation

The results signify that *E. crassipes* leaves extract can be utilized as organic fertilizer for seed germination and seedling growth along with enhancement in nutrient amount of soil. Through this study, it is concluded that germination is found to be highest in block where extract was applied in 25:75 (Water hyacinth extract: distilled water) ratio, i.e. 25:75 concentration in both species. Highest average diameter and height was observed in 25:75 in *P. roxburghii* whereas number of leaves was found highest in 75:25 concentration. In the case of *B. purpurea*, highest diameter, height and number of leaves was observed in 75:25, 50:50 and 25:75 concentration respectively. Germination as well as growth of seedlings was found least in the case of control. It was found that pH is decreasing with increase in concentration of extract in mycorrhiza mixed soil used for *P. roxburghii* whereas pH is decreasing negligibly by 0.03 in the case of normal soil. It was found that there is increase in N, P, K with increase in concentration of extract in both soils.

Extract can be used as fertilizer specific crop or plant species in agroforestry crops. This might replace use of expensive inorganic fertilizers in forest nursery work. Extract can be used as fertilizer in soil with poor amount of N, P, K and C value. Similar studies should be done to find out effects of rhizome extract of *E. crassipes* on species found in Nepal. Effect of extract should be studied on species found in tropical and temperate region where *E. crassipes* infestation is seen to prevent extra cleaning cost.

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