

Quantitative Estimation of Phytochemicals Present in Selected Weeds and Their Allelopathic Effect on Wheat (*Triticum aestivum* L.) Seedlings at Paklihawa, Nepal

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Abstract

Leaves extracts of the selected weeds (*Parthenium hysterophorus*, *Lantana camara*, *Artemisia vulgaris* and *Achyranthes aspera*) were screened quantitatively for their phytochemical constituents and their allelopathic potential against the germination and seedling growth of wheat at Paklihawa campus. Alkaloids, flavonoids and saponins content were determined quantitatively using gravimetric method. The results showed highest alkaloid in *Lantana* (18.01%), flavonoid content in *Parthenium* (13.63%) and saponin content in *Artimisia* (16.23). Allelopathic effects of extract on germination, coleoptile length, shoot and root length, weight of biomass and root shoot ratios were studied. Laboratory based experiment showed that with the increasing concentration of selected botanicals, the germination percentage, seedling length and seedling weight of wheat were significantly decreased while mild concentration of *Achyranthes aspera* leaf extract enhance the seedling growth.

Keywords: Allelopathic, Gravimetric, Screening, Weeds

Introduction

Nepal is an agrarian country serving the livelihood of 65.6% of total Nepalese population. Agriculture sector contributes 27% to the national GDP (MOALD, 2021). Wheat (1,736,849 Mt.) is the third most important cereal after rice (4,299,079 Mt.) and maize (2,231,517 Mt.), contributing 20% of the total cereal production in Nepal (MOAD, 2017). Wheat yields suffer from some factors such as lack of reliable irrigation, unsuitable weather, incidence of disease, various weeds and lack of improved technology (NWRP, 2011). Agricultural researchers have found that weeds cause 17-25% losses in wheat annually due to their competitive and allelopathic nature (Shad, 1987). Phytochemicals are naturally occurring compounds found in vegetables, fruits, medicinal plants, aromatic plants, leaves, flowers and roots, and are grouped as primary (Carbohydrates, proteins and lipids) and secondary (polyphenols, steroids, alkaloids etc.) metabolites based on their function in plant metabolism (Bargah, 2017). Secondary metabolites have no direct role in the growth and development of plant but have evolved in the defense against biotic and abiotic stresses. Their distributions vary both

qualitatively and quantitatively depending on age and developmental stage of plants (Harbone, 1972).

A large number of allelochemicals, which are released by plants are stimulatory or have inhibitory effects with the interactions of weeds and crops (Burhan & Shaukat, 2000). Allelochemicals released from leaves, stem roots and other plant parts have capacity to control various weeds (Nasrine, 2011). Nevertheless, leaves seem to be the most consistent sources of chemicals involved in phytotoxicity (Reinhardt & Bezuuidenhout, 2001). As the leaf is the most metabolically active plant body, it is reasonable to believe that it introduces a greater diversity of allelochemicals and, hence, greater allelopathic effects (Riberio et.al., 2009).

Any direct or indirect, harmful or beneficial effect of one plant as a donor plant on another as a recipient plant through the production of chemical compounds that escape into the environment is called allelopathy. Allelopathy and autotoxicity can play significant roles under both natural and manipulated ecosystems, mainly by adversely affecting seed germination and seedling growth (Rice, 1984) whereas one of the main advantages of allelochemicals is the discovery of new modes

of action for the development of bio-herbicides (Macías, et al., 2003). Therefore, a better weed management is required, where allelopathy can play an effective role in the crop field system.

The present study was done to quantify alkaloids, flavonoids and saponins present in *Parthenium hysterophorus*, *Lantana camara*, *Artemisia vulgaris* and *Achyranthus aspera* and assess their allelopathic effect in germination and seedling growth of wheat at Paklihawa condition.

Materials and Methods

Quantitative phytochemicals screening and allelopathic effect of *Parthenium hysterophorus*, *Lantana camara*, *Artemisia vulgaris* and *Achyranthus aspera* extracts upon the seed germination and seedling growth of wheat cultivar were conducted at Institute of Agriculture and Animal science, Paklihawa Campus. Sample plants were collected around the vicinity of campus. Leaves and tender stem were separated from other parts of plants and were thoroughly cleaned and spread in open area for shade drying for few hours followed by air drying at room temperature ($25\pm 5^{\circ}\text{C}$) for 8-10 days. The dried plant parts were then grinded using electrical mixture into fine powder form.

Quantitative phytochemical analysis

The powdered part was then used to quantify the presence of alkaloids, flavonoids and saponins as discussed below.

Quantitative estimation of alkaloid: Alkaline precipitation gravimetric method based on Harbone (1972) was used for the determination of alkaloid content. 5gm of dried sample was weighed and wetted with 50 ml of 10% acetic acid solution and allowed to stand for 4 hours after thoroughly shaking. The mixture was then filtered through Whatman No. 42 filter paper. Filtrate was evaporated by using water bath until the volume reduces to one quarter of its original. By drop wise addition of Ammonium Hydroxide (NH_4OH), alkaloid in the extract was precipitated. Precipitate was recovered by filtration using a pre weighed wetted filter paper

and washed with 1% NH_4OH . It was dried in the oven at 80°C for an hour which was cooled in desiccator and reweighed.

Weight of alkaloid was determined by using formula (Harbone, 1972).

$$\text{Alkaloid \%} = \frac{W_2 - W_1}{W_1} \times 100$$

Where,

W = weight of sample

W1 = weight of empty filter paper

W2 = weight of paper + alkaloid precipitate

Quantitative estimation of saponin: Double solvent extraction gravimetric method as explained by (Harbone, 1984) was used to determine the saponin content of sample. Five grams (5gm) of powdered sample was weighed and mixed with 50 ml of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in water bath for 90 minutes at 55°C . It was filtered through Whatman No. 42 filter paper. The residue was extracted with 50ml of the 20% ethanol and both extracts were pooled together. The combined extract was reduced to about 40mls at 90°C and transferred to a separating funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was done by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partition was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted with 60ml of normal butanol. The combined extracts were washed twice with 10ml of 5% aqueous NaCl solution and evaporated to dryness in a pre-weighed evaporating dish. The saponin content was determined using the following formula given by (Harbone, 1972).

$$\% \text{ of saponin} = \frac{W_2 - W_1}{W} \times 100$$

W = Weight of sample

W1 = Weight of empty evaporating dish

W2 = Weight of dish + saponin content

Quantitative estimation of flavonoid: Flavonoid determination was by the method reported by Ejikeme, et al., 2014 and Boham & Kocipai-Abyazan, 1974. Exactly 50 cm^3 of 80% aqueous methanol added was added to 2.50 g of sample in a 250 cm^3 beaker, covered, and allowed to stand

for 24 hours at room temperature. After discarding the supernatant, the residue was reextracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each wood sample. Each wood sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained.

Flavonoid %= Weight of flavonoid/weight of sample x 100.

Allelopathic test

50 grams of crude powder of each collected samples (*Artemisia vulgaris*, *Parthenium hysterophorus*, *Lantana camera*, *Achyranthus aspera*) were soaked in 250 ml of distilled water separately and macerated overnight (12 hrs) in percolator. Mixture was then filtered through Whatman filter paper no. 42. The filtrate was boiled for five minutes in a heating mantle then allowed to cool by keeping in desiccator. The solution was kept in deep freezer at 4°C for future use. This solution was regarded as stock solution.

Wheat was taken as a sample plant to observe the allelopathic effect of extracts of four plants. Wheat seeds were collected from the Lumbini Agro-vet Bhairahwa, Rupandehi. The aqueous extracts of *Parthenium*, *Artmisia*, *Lantana*, and *Achyranthus* at different concentrations were prepared to observe allelopathic effect.

The surface was sterilized by ethanol (70% alcohol). Two seeds were placed on each polybag containing soil collected from Horticulture farm of the campus. Similar setup was done by (Goda, 1987). Altogether 13 treatments were made and distilled water as control. Each treatment was maintained as stock solution, 10% and 25% of prepared stock solution. The setup was replicated thrice. Experiment was set on 22 November 2019 in Completely Randomized Design (CRD). Each polybag was irrigated with 20

ml botanical extract up to 15 days in alternate days. The numbers of germinated seeds were recorded in a day interval. Shoot and coleoptile length were measured after 5 and 9 days of experimental setup respectively in a day interval up to 15 days. The data means were then accordingly subjected to Analysis of Variance (ANOVA) individually using R-stat and means were separated by DMRT (Duncan's Multiple Range Test) to identify significant differences.

T1	T13	T4
T2	T12	T13
T3	T11	T5
T4	T10	T12
T5	T9	T10
T6	T8	T9
T7	T7	T1
T8	T6	T6
T9	T5	T3
T10	T4	T11
T11	T3	T2
T12	T2	T7
T13	T1	T8

Figure 2: Research design

Notation of Treatments:

Stock solution of *Parthenium* -T1

10% of stock solution of *Parthenium*-T2

25% of stock solution of *Parthenium*-T3

Stock solution of *Lantana*-T4

10% of stock solution of *Lantana*-T5

25% of stock solution of *Lantana*-T6

Stock solution of *Artimisia vulgaris*-T7

10% of stock solution of *Artimisia vulgaris*-T8

25% of stock solution of *Artimisia vulgaris*-T9

Stock solution of *Achyranthes aspera*-T10

10% of stock solution of *A. aspera*-T11

25% of stock solution of *A. aspera*-T12

Control/Distilled water-T13

Results and Discussions

Quantitative Estimation of Secondary Metabolites

Quantitative analysis of phytochemicals was carried out by using gravimetric method which revealed that *L. camara* contained highest percentage of alkaloid (18.01) among all the tested botanicals. The result was quite similar to observations of (Bhuvanewari and Giri, 2018).

Table 1: Quantitative estimation of alkaloid, flavonoid and saponin present in weeds collected at Paklihawa Campus, 2019

Plant Extract	Alkaloid%	Flavonoid%	Saponin%
<i>P. hysterophorus</i>	17.78 ^a	13.63 ^a	5.76 ^d
<i>L. camara</i>	18.01 ^a	7.51 ^b	13.65 ^b
<i>A. vulgaris</i>	6.78 ^c	2.85 ^d	16.29 ^a
<i>A. aspera</i>	16.71 ^b	5.89 ^c	11.55 ^c
Grand mean	14.82	7.47	11.81
CV	3.30	6.03	6.04
SEM	1.41	1.19	1.18
LSD	0.92***	0.84***	1.34***

Note: LSD: Least significant difference, CV: Coefficient variation, SEM: Standard error of mean
Different alphabets signify significant difference between the treatments.

*Significant at 5% level of significance

**Significant at 1% level of significance

***Significant at 0.1% level of significance

Similarly, flavonoid content was found to be highest in *P. hysterophorus* (13.63) and lowest in *A. vulgaris* (3.85). Pradhan and Sarangdevot (2020) reported that *P. hysterophorus* contain 5.25% flavonoids. Such variation in the quantity of metabolites may be due to ecological or geographical differences, plant parts used, plant age, solvent used and other unseen factors (Bourgaud et al., 2001).

Similarly, quantitative test of tested botanicals for Saponin content revealed that *A. vulgaris* contained highest percentage of saponin (16.29) followed by *Lantana camara* and *A. aspera* respectively but *P. hysterophorus* contained very trace amount of saponin (5.75). Not all the phytochemicals were present in all plant parts and that percent occur in different degree based on type of extracting solvent used (Tijjani, et al., 2009). There were only limited researches have been done for the quantitative

determination of metabolites for other tested botanicals.

Allelopathic effect of *Parthenium*, *Lantana*, *A. vulgaris* & *A. aspera* on wheat seedlings

While analyzing the effect of different botanical extract on seed germination of wheat, average seed germination at 4 and 5 days after treatment setup were 1.13 and 1.15 respectively. Maximum seed germination was observed at T12 (i.e. 25% stock solution of *Achyranthes aspera*) as 2 which was at par with all other treatments except T1, T2, T4, T6, & T7. Though, the effect on seed germination was found to be significantly different with each other. Detail is shown in table 2 below.

Table 2: Effect of extracts in seed germination of wheat

Treatment	No. of seed germination 4 DAT	No. of seed germination 5 DAT
T ₁	0.00 ^d	0.00 ^c
T ₂	0.33 ^{cd}	0.67 ^{bc}
T ₃	1.33 ^{abc}	1.67 ^{ab}
T ₄	0.67 ^{bcd}	0.67 ^{bc}
T ₅	1.00 ^{abcd}	1.00 ^{abc}
T ₆	0.67 ^{bcd}	0.67 ^{bc}
T ₇	0.67 ^{bcd}	0.67 ^{bc}
T ₈	1.67 ^{ab}	1.33 ^{ab}
T ₉	1.33 ^{abc}	1.33 ^{ab}
T ₁₀	1.67 ^{ab}	1.67 ^{ab}
T ₁₁	1.67 ^{ab}	1.67 ^{ab}
T ₁₂	2 ^a	2.00 ^a
T ₁₃	1.67 ^{ab}	1.67 ^{ab}
Grand mean	1.13	1.15
CV	51.17	50.67
SEM	0.12	0.12
LSD	0.97**	0.97**

Note: ns: non-significant, LSD: Lower significant difference, DAT: Days after treatment, different alphabets signify significant difference between the treatments

*Significant at 5% level of significance

**significant at 1% level of significance

***significant at 0.1% level of significance

Among all the sample of botanicals, stock solution of *P. hysterophorus* possess higher negative allelopathic effect on germination of wheat seed where as 25% of stock solution of *A. aspera* promoted the seed germination. (Maharjan, et al., 2007) also reported the inhibitory effect of *P. hysterophorus* on germination of many crops such as wheat, barley,

maize etc. Inhibitory effect was found to be higher with increasing concentration with plant extract.

While analyzing the effect of different botanical extract on coleoptile length of wheat seedlings, average coleoptile length at 9 days after treatment setup was 2.98cm. Maximum length was observed at T12 (i.e. 25% stock solution of *Achyranthes aspera*) as 3.5cm which was at par with all other treatments except T7 & T4 where coleoptile length was only 2.37cm & 2.63cm respectively (Table 3). Though, the effect on coleoptile length was not found to be significantly different with each other.

Average coleoptile length at 11 days after treatment setup was 3.12cm. Maximum length was observed at T12 (i.e. 25% stock solution of *Achyranthes aspera*) as 3.67cm which was at par with all other treatment except T6 & T7 where coleoptile length was only 2.70cm & 2.33cm respectively. The effect of different botanicals extracts on coleoptile length at 11 days after treatment setup was found to be significant at 5% level of significance (Table 3).

Table 3: Effects of extracts in coleoptile length of wheat seedlings

Treatment	Coleoptile length 9 DAT	Coleoptile length 11 DAT	Coleoptile length 13 DAT
T ₁	2.93 ^{ab}	3.06 ^{abc}	3.10 ^{ab}
T ₂	3.00 ^{ab}	3.47 ^{ab}	3.53 ^{ab}
T ₃	3.0 ^{abc}	3.03 ^{abc}	3.17 ^{ab}
T ₄	2.63 ^{ab}	3.17 ^{abc}	3.17 ^{ab}
T ₅	2.83 ^{ab}	2.87 ^{abc}	2.87 ^{bc}
T ₆	2.83 ^{ab}	2.70 ^{bc}	2.83 ^{bc}
T ₇	2.37 ^b	2.33 ^c	2.33 ^c
T ₈	2.97 ^{ab}	3.07 ^{abc}	3.10 ^{ab}
T ₉	3.13 ^{ab}	3.23 ^{ab}	3.33 ^{ab}
T ₁₀	3.27 ^a	3.33 ^{ab}	3.33 ^{ab}
T ₁₁	3.03 ^{ab}	3.10 ^{abc}	3.10 ^{ab}
T ₁₂	3.43 ^a	3.67 ^a	3.67 ^a
T ₁₃	3.17 ^{ab}	3.53 ^{ab}	3.53 ^{ab}
Grand mean	2.98	3.12	3.15
CV	14.23	14.59	12.41
SEM	0.07	0.08	0.08
LSD	0.71 ^{ns}	0.77 [*]	0.66 [*]

Note: ns: non-significant, LSD: Lower significant difference, DAT: Days after treatment, different alphabets signify significant difference between the treatments

*Significant at 5% level of significance

**significant at 1% level of significance

***significant at 0.1% level of significance

Average coleoptile length at 13 days after treatment setup was 3.14cm. Maximum length was observed at T12 (i.e. 25% stock solution of *Achyranthes aspera*) as 3.67cm which was at par with all others treatment except T5, T6 & T7 where coleoptile length was only 2.87cm, 2.83cm & 2.33cm respectively. The effect of different botanicals extracts on coleoptile length at 12 days after treatment setup was found to be significant at 5% level of significance.

Above result showed that stock solution of *A. vulgaris* had strong inhibitory effect on coleoptile of wheat than other tested plant extracts. The result was also supported by findings of (Eom et.al., 2006) which revealed that the volatiles are potent inhibitors of seedlings growth and germination. Higher concentrations of aqueous extract of *A. vulgaris* not only inhibit wheat germination but also their shoot and root growth (El-Fattah, 2011). Low concentration of *A. aspera* extract promotes the coleoptile length of wheat which corresponded with the experimental result of (Khan & Shaukat, 2006).

While analyzing the effect of different botanical extracts on shoot length of wheat seedlings, average shoot length at 5 days after treatment setup was 4.62cm. Maximum length was observed at T13 (i.e., control/distilled water) as 5.77cm which was at par with all others treatments except T1 (i.e. stock solution of Parthenium), T5 (i.e. 10% stock solution of Lantana) and T7 (i.e. stock solution of *Artimisia vulgaris*) where shoot length was observed only 0.75cm, 4.20cm and 3.12cm respectively. The effect of different botanicals extracts on shoot length at 5 days after treatment setup was found to be significant at 0.1% level of significance.

Average length at 7 days after treatment setup was 11.88cm. Maximum length was observed at T12 (i.e., 25% stock solution of *Artimisia vulgaris*) as 14.00cm which was at par with all others treatments except T1 (i.e., stock solution of Parthenium), T5 (i.e., 10% stock solution of Lantana) and T7 (i.e., stock solution of *Artimisia vulgaris*) where shoot length was observed only 2.93cm, 10.47cm & 10.37cm respectively. Though, the effect on shoot length at 7 days after treatment setup was found to be significant at 0.1% level of significance.

Table 4: Effect of extracts in shoot length of wheat seedlings

Treatment	Shoot length 5 DAT	Shoot length 7 DAT	Shoot length 9 DAT	Shoot length 11 DAT	Shoot length 13 DAT	Shoot length 15 DAT
T ₁	0.75 ^d	2.93 ^d	7.70 ^b	11.53 ^b	13.10 ^b	16.63 ^b
T ₂	5.37 ^{ab}	12.93 ^{abc}	19.80 ^a	22.93 ^a	23.13 ^a	25.37 ^a
T ₃	5.20 ^{ab}	12.83 ^{abc}	19.27 ^a	22.20 ^a	23.10 ^a	26.37 ^a
T ₄	5.10 ^{ab}	12.73 ^{abc}	19.70 ^a	23.20 ^a	23.77 ^a	24.53 ^{ab}
T ₅	4.20 ^{bc}	10.47 ^{bc}	16.40 ^a	18.13 ^a	18.20 ^{ab}	19.80 ^{ab}
T ₆	4.50 ^{ab}	11.67 ^{abc}	17.33 ^a	18.0 ^a	18.27 ^{ab}	19.47 ^{ab}
T ₇	3.12 ^c	10.37 ^c	17.00 ^a	20.1 ^a	20.17 ^a	22.57 ^{ab}
T ₈	4.97 ^{ab}	12.93 ^{abc}	19.73 ^a	22.3 ^a	22.30 ^a	24.17 ^a
T ₉	5.23 ^{ab}	13.17 ^{abc}	17.80 ^a	21.27 ^a	21.27 ^a	22.93 ^{ab}
T ₁₀	4.90 ^{ab}	13.03 ^{abc}	19.77 ^a	22.77 ^a	22.87 ^a	24.53 ^a
T ₁₁	5.27 ^{ab}	13.53 ^{ab}	20.67 ^a	23.3 ^a	23.30 ^a	24.10 ^a
T ₁₂	5.67 ^a	14.00 ^a	21.33 ^a	24.57 ^a	24.57 ^a	25.43 ^a
T ₁₃	5.77 ^a	13.90 ^a	21.27 ^a	24.13 ^a	24.23 ^a	25.03 ^a
Grand mean	4.62	11.88	18.29	21.11	21.40	23.15
CV	15.69	13.33	15.26	17.11	16.35	15.50
SEM	0.23	0.12	0.67	0.56	0.69	0.78
LSD	1.22***	2.66***	4.71***	6.08*	5.89*	6.05*

Note: ns: non-significant, LSD: Lower significant difference, DAT: Days after treatment, different alphabets signify significant difference between the treatments

*Significant at 5% level of significance

**significant at 1% level of significance

***significant at 0.1% level of significance

Average length at 9 days after treatment setup was 18.29cm. Maximum length was observed at T₁₂ (i.e. 25% stock solution of *Achyranthes aspera*) as 21.33cm which was at par with all others treatments except T₁ (i.e. stock solution of *Parthenium*) where shoot length was observed only 7.70cm. Though, the effect on shoot length at 9 days after treatment setup was found to be significant at 0.1% level of significance.

Average length at 11 days after treatment setup was 21.11cm. Maximum length was observed at T₁₂ (i.e. 25% stock solution of *Achyranthes aspera*) as 24.57cm which was at par with all others treatments except T₁ (i.e. stock solution of *Parthenium*) where shoot length was observed only 11.53cm. Though, the effect on shoot length at 11 days after treatment setup was found to be significant at 5% level of significance.

Average length at 13 days after treatment setup was 21.40cm. Maximum length was observed at T₁₂ (i.e., 25% stock solution of *Achyranthes aspera*) as 24.57cm which was at par with all others treatments except T₁ (i.e., stock solution of *Parthenium*) where

shoot length was observed only 13.10cm. Though, the effect on shoot length at 12 days after treatment setup was found to be significant at 5% level of significance.

Average length at 15 days after treatment setup was 23.15cm. Maximum length was observed at T₁₂ (i.e., 25% stock solution of *Achyranthes aspera*) as 25.43cm which was at par with all others treatments except T₁ (i.e., stock solution of *Parthenium*) where shoot length was observed only 16.63cm. Though, the effect on shoot length at 16 days after treatment setup was found to be significant at 5% level of significance.

Leaf extract of *P. hysterothorus* had strong inhibitory effect on shoot length of wheat as compared to other treatment. Similar result was also found by (Khan et.al., 2005) in inhibition of shoot and root growth by allelochemicals released from *P. hysterothorus*. Likewise, shoot length was promoted by 25% of stock solution of *A. aspera* which is in accordance with research of Khan and Shaukat (2006).

Table 5: Effect of extracts in root length. Root shoot ratio and change in weight

Treatment	Root length 15 DAT	Change in weight	Root Shoot ratio 15 DAT
T ₁	5.47 ^b	0.22 ^{ab}	0.34 ^a
T ₂	10.37 ^{ab}	0.29 ^{ab}	0.41 ^a
T ₃	9.80 ^{ab}	0.27 ^{ab}	0.37 ^a
T ₄	6.43 ^{ab}	0.29 ^{ab}	0.25 ^a
T ₅	7.57 ^{ab}	0.24 ^{ab}	0.38 ^a
T ₆	7.50 ^{ab}	0.17 ^b	0.38 ^a
T ₇	7.03 ^{ab}	0.27 ^{ab}	0.31 ^a
T ₈	8.93 ^{ab}	0.33 ^a	0.37 ^a
T ₉	6.50 ^{ab}	0.30 ^{ab}	0.29 ^a
T ₁₀	11.33 ^a	0.27 ^{ab}	0.46 ^a
T ₁₁	9.40 ^{ab}	0.32 ^a	0.39 ^a
T ₁₂	8.63 ^{ab}	0.32 ^a	0.34 ^a
T ₁₃	8.37 ^{ab}	0.19 ^{ab}	0.33 ^a
Grand mean	8.25	0.27	0.36
CV	34.24	26.15	31.88
SEM	0.45	0.01	0.01
LSD	4.76 ^{ns}	0.12 ^{ns}	0.19 ^{ns}

Note: ns: non-significant, LSD: Lower significant difference, DAT: Days after treatment, different alphabets signify significant difference between the treatments

*Significant at 5% level of significance

**significant at 1% level of significance

***significant at 0.1% level of significance

On analyzing the effects of different botanical extracts on root length of wheat seedlings, average root length at 15 days after treatment setup was found to be 8.25. Maximum root length was observed at T10 (i.e. stock solution of *Achyranthes aspera*) as 11.33cm which was at par with all others treatments except T1 (i.e. stock solution of *Parthenium*) where the root length was observed only 5.47 cm. Though, the effect on root length was not found to be significantly different with each other.

Similarly, average change in weight on fresh weight of biomass with dry weight of biomass after the experimental setup was found to be 0.27g. Maximum weight was observed at T8 (i.e.10% stock solution of *Artimisia vulgaris*) as 0.33g which was at par with all other treatments except T6 (i.e. 25% stock solution of *Lantana*) where the weight was found only to be 0.17g. However, the effect of botanicals on weight of wheat seedlings was not found to be significantly different with each other.

Likewise, the average root shoot ratio after the cessation of experimental setup was 0.36. Maximum

ratio was observed at T10 (i.e. stock solution of *Achyranthes aspera*) as 0.46 whereas minimum root shoot ratio was observed at T9 (25% stock solution) as 0.29. Though, the effect of botanical extract on root shoot ratio was not found to be significantly different with each other.

Root length of wheat was strongly inhibited by the aqueous extract of *P. hysterophorus* which coincided with the findings of (Rashid, et al., 2008). Likewise, change in weight was relatively decreased by the leaf powder of *L. camara* while it was increased by *A. vulgaris* extract. Root shoot ratio was lowered by leaf extract of *L. camara* and higher was found by leaf extract of *A. vulgaris*.

Conclusion

Quantitative test of phytochemical screening done at Paklihawa Campus revealed that alkaloid content was highest in *Lantana camera* (18.01%), flavonoid content in *Parthenium* (13.63%) and saponin content in *Artimisia* (16.23%). Diluted concentration (25% of stock solution) of *Achyranthes aspera* leaf extract showed the potentiality of being a growth promoter for wheat or as in other crops of same family as germination percentage was seen maximum. Similarly, application of *Parthenium* extract shows the highest inhibitory effect against the shoot and root length in wheat seedling. *L. camara* greatly reduced the coleoptile length of wheat seedlings so it can be said that these plants around agricultural field may affect growth parameter of wheat. Further studies on characterization of phytochemicals and their specific role in different agricultural crops is important. In this regard, a further study on characterization of phytochemicals and their specific role in different agricultural crops is to be prioritized.

Author Contributions

All the authors were involved in developing the concepts, research design and reviewing of relevant literatures. Pravin Budhathoki, Manoj Mandal and Amita Gyawali collected the plants, performed the research and prepared the initial manuscript. Subodh Khanal generated and provided the conceptual

framework, analyzed the data and prepared the final manuscript.

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References

- Bargah, R. K. (2017). Preliminary Phytochemical screening analysis and therapeutic potential of *Tecoma stans* (L.). *International Journal of Applied Chemistry*, 13(1), 129-134.
- Bhuvanewari, E., & Giri, R. S. (2018). Physicochemical and phytochemical screening in *Lantana camara* leaves. *Journal of Pharmacognosy and Phytochemistry*, 7(6), 1962-1966.
- Boham, B. A., & Kocipai-Abyazan, R. (1974). Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific sci*, 48(4), 458-463.
- Bourgau, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: a historical perspective. *Plant science*, 161(5), 839-851.
- Burhan, N., & Shaukat, S. (2000). Effects of atrazine and phenolic compounds on germination and seedling growth of some crop plants. *Pakistan Journal of Biological Sciences*, 3(2), 269-274.
- El-Fattah, A. (2011). Allelopathic effects of *Artemisia princeps* and *Launae sonchoids* on rhizospheric fungi and wheat growth. *African Journal on Microbiology Research*, 5(4), 419-424.
- Eom, S., Yang, S., & Westom, L. (2006). An evaluation of the allelopathic potential of selected perennial groundcovers: foliar volatiles of catmint (*Nepeta × faassenii*) inhibit seedling growth. *Journal of Chemical Ecology*, 32(8), 1835-1848.
- Ejikeme, C., Ezeonu, C. S., & Eboatu, A. N. (2014). Determination of Physical and Phytochemical Constituents of some Tropical Timbers Indigenous to Nigerdelta area of Nigeria. *European Scientific Journal*, 10(18), 247-270.
- Goda, S.E. (1987). *Germination of Acacia nilotica seeds* (pp. 4). University Press.
- Harbone, J. B. (1984). *Phytochemical methods*, (3rd ed.) (pp. 21-29). Chapman and Hall.
- Harbone, J. (1972). Phytochemical ecology. *Annual proceeding of the Phytochemical Society*. doi: <https://doi.org/10.1002/food.19730170231>
- Khan, D., & Shaukat, S. (2006). Phytotoxic potential of *Achyranthes aspera* L.: A tropical medicinal weed of Pakistan. *International Journal of Biotechnology*, 3, 57-71.
- Khan, M., Marwat, K., Gul, H., & Zahid, H. (2005). Bioherbicidal effects of tree extracts on seed germination and growth of crops and weeds. *Pakistan Journal of Weed Science and Research*, 11(3/4), 179-184.
- Macías, F., Marin, D., Oliveros-Bastidas, A., Varela, R., Simonet, A., Carrera, C., & Molinillo, J. (2003). Allelopathy as a new strategy for sustainable ecosystems development. *Biological Sciences in Space*, 17(1), 18-23.
- Maharjan, S., Shrestha, B.B., & Jha, P.K. (2007). Allelopathic effects of aqueous extract of leaves of *Parthenium hysterophorus* L. on seed germination and seedling growth of some cultivated and wild herbaceous species. *Sci World*, 5(33).
- MOAD. (2017). *Statistical information on Nepalese agriculture 2015/2016*. Sinhadurbar, Kathmandu: Agri Statistics Section, Monitoring, Evaluation and Statistics Division, Ministry of Agricultural Development.
- MOALD. (2021). *Ministry of Agriculture and Livestock Development*. Retrieved Jan 30, 2021, from <https://www.moald.gov.np/ministry-info>
- Nasrine, S. (2011). *Allelochemicals from some medicinal and aromatic plants and their potential*

- use as bioherbicides. (Unpublished Doctoral dissertation), Université Badji-Mokhtar, Annaba.
- NWRP. (2011). *Annual report 2010/11*. National Wheat Research Program. Bhairahawa, Nepal.
- Pradhan, P., & Sarangdevot, Y.S. (2020). Estimation of total phenols and flavonoids content in *Parthenium hysterophorus* aerial parts. Retrieved 1,30,2021, from <https://www.semanticscholar.org/paper/ESTIMATION-OF-TOTAL-PHENOLS-AND-FLAVONOIDS-CONTENT-Pradhan-Sarangdevot/19b7fac9af31f6292b26d5f9602ffb1167529c33>
- Rashid, H., Khan, M., Amin, A., Nawab, K., Hussain, N., & Bhoumik, P. (2008). Effect of *Parthenium hysterophorus* L., root extracts on seed germination and growth of maize and barley. *Ameicas Journal of Plant Science and Biotechnology*, 2(2), 51-55.
- Reinhardt, C., & Bezuuidenhout, S. (2001). Growth stage of *Cyperus esculentus* influences its allelopathic effect on ectomycorrhizal and higher plant species. *Journal of Crop Production*, 4(2), 323-333.
- Riberio, R., Machado, E., Santos, M., & Oliveria, R. (2009). Seasonal and diurnal changes in photosynthetic limitation of young sweet orange trees. *Environmental and experimental botany*, 66(2), 203-211.
- Rice, E. (1984). *Allelopathy* (2nd ed.). Academic Press, USA.
- Shad, R. (1987). *Status of weed science activities in Pakistan*. Progressive Farming.
- Tijjani, M., Bello, I., Aluyu, A., Olurische, T., Maidawa, S., Habila, J., & Balogun, E. (2009). Phytochemical and antibacterial studies of root extract of *Cochlospermum tinctorium*. *Research Journal of Medicinal Plants*, 3, 16-22.