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Table: -, Figs: 10

Herbicidal effects of *Withania somnifera* L. leaf extracts on *Cannabis sativa* L., *Hordeum vulgare* L. and *Cicer arietinum* L.

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ABSTRACT

We identified the phytochemicals in *Withania somnifera* L, a multipurpose medicinal plant of the Himalayan región using TLC, FTIR and HPLC. Eleven formulations were made by mixing in different ratios of *Withania* leaf extract, wood ash and distilled wáter. Wood chips and twigs of 3-years old *Pinus roxburghii* tree were completely burnt till ashes was used as Wood ash. The 11-formulationss were i.e. formulation I (Withania leaf extract 100%)), formulation II (75:25 concentration (Withania leaf extract: Distilled water)), formulation III (50:50 concentration (Withania leaf extract: Distilled water), formulation IV (25:75 concentration (Withania leaf extract: Distilled water)), formulation V (75:25 concentration (Withania leaf extract: Wood ash)), formulation VI (50:50 concentration (Withania leaf extract: Wood ash)), formulation VII (25:75concentration (Withania leaf extract: Wood ash)), formulation VIII (Wood ash 100%)), formulation IX (75:25 concentration (Wood ash: Distilled water)), formulation X (50:50 concentration (Wood ash:Distilled water)), formulation XI (25:75concentration (Wood ash: Distilled water)). The herbicidal activity of formulations was earlier studied against *Cannabis sativa* L. (banned narcotic plant). Their herbicidal activity was tested on seeds germination and seedlings growth of *Cannabis sativa* L weed and 2 crops: *Hordeum vulgare* L and *Cicer arietinum* L. In Pot culture, the formulation V spray caused maximum reduction in root length of *Cannabis* (53 %) > *Hordeum* (23 %) and *Cicer* (22 %) than control. The formulations were also tested on crops for their herbicidal effects. In Petri plate bioassy, the formulation V (75:25 concentration (Withania leaf extract: Wood ash) showed maximum reduction in seed germination and seedling growth of *C. sativa* weed, but had little effect on growth of test crops. The reduction in stem length was maximum (55 %) in *Cannabis* > *Hordeum* (20 %) > *Cicer* (19 %) than control. Glyphosate decreased the stem length of *Cicer* (80 %) > *Hordeum* (58 %) and *Cannabis* (16 %) over control. While the reduction in root length was in *Cicer* (75 %) > *Hordeum* (73 %) > *Cannabis* (18 %) than control. The root and stem extract formulations were more phytotoxic to *Cannabis sativa*. Formulation V (75:25 concentrations (Withania leaf extract: wood ash)) reduced the number of leaves, number of shoot, root branches and stem and root length of *Cannabis sativa*.

Keywords: Allelopathy, *Cicer arietinum*, glyphosate, *Hordeum vulgare*, pine, *Pinus roxburghii*, seeds germination, weeds, *Withania somnifera*, withanolides, wood ash

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INTRODUCTION

Weeds are unwanted intruders into crop fields, they compete for growth resources (wáter, light, nutrients and space) abd decrease crops yields (3). The invasive weeds have short seed dormancy, high seed germination, rapid growth, high reproductivity, short life span, and very high environmental plasticity, which makes them dominant world wide (4). These are threats to native diversity. *Cannabis sativa* L. is prominent invasive specie in Himalayan region (18). It is prohibited and banned plant due to use as narcotic Most resource poor farmers adopt manual weed-control methods viz., hand pulling and hand-hoeing (12), but due to unavailability of labour some farmers use herbicides (13). Use of synthetic herbicides causes various problems *viz.*, residual toxicity, reduction choice of succeeding crops, development of herbicide resistant weeds and source for carcinogenic and mutagenic effects on human beings and animals (16).

(A). ***Withania somnífera***

(B). ***Cannabis sativa***

Figure 1. (A). *Withania somnifera* L and (B). *Cannabis sativa* L growing in our University Nursery

Allelopathy is a mechanism in which a plant adversely affects the seed germination and growth of another plant (19) to establish its Pure stand. Medicinal plants are rich in allelochemicals and these allelochimicals may act as herbicides for weed control (14). Allelochemicals are secondary metabolites [phenolic acids, coumarins, flavonoids,

terpenoids, alkaloids and sulfides (9,31) produced via several metabolic plant pathways. They affect all basic plant processes such as photosynthesis, chlorophyll production, respiration, hormonal balance, protein synthesis and plant water relations (23). They enter the soil through rain leachates from foliage, root exudation and decomposition of plant residues or as microbial by-products of residue decomposition, and selectively inhibit the growth of other plants and soil microorganisms (16). Originally classified as waste products, allelochemicals have been extensively investigated by ecologists and pharmacologists as they participate in many complex biological processes (21). Allelopathic potential of plants can be used to control pathogens and weeds (6). Allelopathic potential of plant species can be exploited in many ways, use of aqueous extracts of plants is one. Different plant species contain allelochemicals in different concentrations (2). Weeds may be better controlled by using the plants that are rich in allelochemicals (13). For better ecological based weed management, the Identification of plant species with greater allelopathic potential and their adverse effects on weeds is required. These plants may be potential sources of allelochemicals to develop natural herbicides (22). An important medicinal plant *Withania somnifera* (L.) Dunal, (Solanaual Fanny) called "Ashwagandha" is rieh in phytochemicals and may have herbicidal role against weeds. It is prominent herb of lower Himalaya (Fig. 1). It has 1250 species. It contains several groups of chemical constituents such as steroidal lactones, alkaloids, flavonoids and tannins (1). At present, more than 12 alkaloids, 40 withanolides and several sitoindosides have been isolated from its weeds shoots, roots and berries (19). Little information is available regarding its phytotoxic effects against weeds. Its extracts suppressive allelopathic potential was evaluated in pot studies on early seedling growth of rice weeds (viz. *Parthenium hysterophorus* L. *Chenopodium album* L and *Achyranthus aspara* L.) to explores its use in weed management. Herbicidal formulations were made with leaf extract, wood ash and distilled water and tested against the weed *Cannabis sativa* and the crops *Cicer arietinum L* and *Hordeum vulgare.* L. This study aimed to evaluate the herbicidal efficiency of *Withania somnifera* (L.) for weed management in Lower Himalaya.

2. MATERIAL AND METHODS

Healthy uniform seeds of *Withania somnifera* L., *Cannabis sativa* L, *Hordeum vulgare ssp. Vulgare* L. 'KB 71' and *Cicer arietinum* L (HC-2') were obtained from YS Parmar University, Nauni. The experiments were conducted in Plant Physiology Laboratory and Nursery área, Shoolini University of Biotechnology and Management Sciences, Solan (Western Himalayas hiils 30°22'44''N to 33° 22'44''N latitude and 75°45'44" E to 79°04'20" E longitude, altitude 1352 m above sea level, mean anual temperatura: 15° C (59° F), Meane summer temperatura: 30°C (86°F), Mean winter temperature: 0°C (32°F). moderate snowfall (0.6 cm) during December and January, mean rainfall: 1413 mm. *W. somnifera* was sown on 9 september, 2017 in nursery area of our University in plastic Pots (12 cm ×25 cm), containing sterilized soil, sand and FYM (1:1:1) in green house at 28/18°C day/night maintained by a combination of heating (16 kW) in night and venting during the day. On 9 April, 2018, at maturity leaves from plants were taken for experimentation.

(i). Leachates: Six months old *Withania somnifera* plants were uprooted from the nursery area. Its stem, roots and leaves were separated, washed, dried in sun for 10

days and weighed separately. Their leachates were prepared separatley by soaking 50 g of each plant part in conical flask for 72 h at 8 ^{o}C in refrigerator for deoxygenation of leaves (25). The aqueous leachates were filtered through 3-layers of muslin cloth and again through one layer of Whatman no.1 filter paper. Leachate prepared in kept for 9 days.

(ii). Extracts: For foliar spray, we prepared 11-aqueous Extracts of dried and powdered *W. somnifera* leaves and Ash (of 3- years old *Pinus roxburghii* plants (Table 1). Leaves of *W. somnifera* were collected before flowering. Sun Dried leaves powder (30 g) was added to 200 ml of Distilled water and kept for 9-days at 22 °C and 60 % humidity in BOD. The extract was filtered like the leachte filtration and diluted to prepare 6-concentrations (Table 1).

Table 1. *Withania* leaf leachate, wood ash extracts prepared for assay.

Treatment	Extract Composition (%)
Extract I	*Withania* leaf extract 100%
Extract II	75:25 concentration (*Withania* leaf extract: Distilled water)
Extract III	50:50 concentration (*Withania* leaf extract: Distilled water)
Extract IV	25:75 concentration (*Withania* leaf extract: Distilled water)
Extract V	75:25 concentration (*Withania* leaf extract: Wood ash)
Extract VI	50:50 concentration (*Withania* leaf extract: Wood ash)
Extract VII	25:75concentration (*Withania* leaf extract: Wood ash)
Extract VIII	Wood ash 100%
Extract IX	75:25 concentration (Wood ash: Distilled water)
Extract X	50:50 concentration (Wood ash: Distilled water)
Extract XI	25:75 concentration (Wood ash: Distilled water)

Wood chips and twigs of 3-years old *Pinus* tree were completely burnt till ashes (burnt it with matchstick), dissolved in Distilled wáter in 1:3 ratio (10 g of ash in 30ml of distilled wáter) and filtered 72 h after soaking. The extracts were stored in tanks until use. These extracts were sprayed with small hand sprayer fitted with nozzle to give fine mist to study their herbicidal effects on *C. sativa*, *Cicer arietinum* and *Hordeum vulgare*.

Bioassays

(i). Petri Plate Bisoassay (Seed germination)

The seeds of test crops [*Cannabis sativa* (L.), *Hordeum vulgare* (L.) and *Cicer arietinum* (L.)] were first soaked in distilled water for 1 h, then surface sterilized with 70 % ethanol for 2 min and rinsed several times with double-distilled water. Six seeds of test crops sown per petridish (100 × 15 Mm) lined with two layers of filter paper and grown for one month in growth chamber. The petriplantes were irrigated every 5- days with 1.0 mL stem, root or leaf extract of *Withania* or wood ashes. Negative and positive controls were glyphosate and distilled water, respectively. Seed germination and seedlings growth (stem and root lengths, and total Dry weight were recorded every 5-days for 30 days.

Germination was recorded daily as per AOSA (1990) until constant count was achieved. Seeds were considered germinated when the radicle and hypocotyl lengths were over 2 mm. The time before 50 % emergence (E50) was calculated as under (21):

$$E_{50} = t_i + \frac{(N/2 - ni)}{n_j - n_i} (tj - t_i)$$

Where, N: Final number of emerged seeds, and ni and nj: Cumulative number of seeds germinated by adjacent counts at times ti and tj, where, ni < N/2 < nj.

Root and shoot lengths were measured with Scale after 14 d. All roots and shoots from each Petri dish were cut separately and oven dried at 70 °C for 48 h to get their dry weight. The seedlings vigour index was calculated as under:

$$SVI = Germination (\%) \times Radicle\ length\ (cm)$$

(ii). Pot Culture (Foliar spray)

The experiments were done in plactic pots (12 cm ×25 cm) in August, 2018. Each pots contained 7 kg sterilized soil, sand and Farm yard manure (FYM) (1:1:1 ratio). The extracts of *Withania somnifera* wood ash and glyphosate were sprayed with small hand sprayer fitted with nozzle to give fine mist on 4 month old test plants (Barley, Chickpea and weed *C. sativa*) grown in pots. The pots were kept in green house (28/18^{o}C day/night by heating (16 kW) in night and venting during the day every 5-days. The experiment was repeated thrice. Morphological features (root length, shoot length, dry weight, number of leaves, number of shoots, number of root branches and leaf área) were determined every 5 days for one month.

Phytochemical analyses

These were done to identify the main groups of chemical constituents present in donor plant. The powdered leaves of *Withania somnifera* were extracted for 72 h with succesive solvents [methanol [1:10 (w/v) ratio with plant material], petroleum ether, chloroform and distilled wáter] separately in soxhlet extractor and discarded the leftover residue. Phytochemical analysis of plant extracts was done asper (19) for carbohydrates, proteins, alkaloids, steroids, terpenoids, saponins, steroids and glycosides. Different phytochemicals in various extracts were identified by precipitation and colour reaction with different reagents.

(i). Thin layer Chromatography (TLC): A drop from extract was loaded on silica gel plate 60 F_{254} (Merck) developed with mobile phase of hexane: chloroform: methanol (50:48:2 v/v). Withanolides were detected in the TLC chromatograms after spraying silica gel plate with Dragendorff's reagent (Fig 2). Withanolides in extract were identified by comparing their Rf values with standard withanolide A, withanolide D and withaferin A purchased from ChromaDex Standards. The Rf values for withanolide A, withaferin A and withanolide D are 0 .58, 0.41 and 0.19, respectively (2).

(ii). Infrared Spectroscopy (IR): The solid extracted compound was observed in FTIR and the peaks confirmed the presence of withanolides in the sample.

(iii). High-performance liquid chromatography: Withanolides (withanolide A, withaferin A and withanolide D) were quantified using a binary pump HPLC system provided by Agilent technologies coupled to a photodiode array detector set at 227 nm. The column was a hypersil BDS C-18 (5 µm, 4.6 x 150 mm). The chromatogram was recorded by EZ-Chrom software. Stock solutions of withanolides (98 % pure ChromaDex) were prepared at a concentration of 0.5 mg/ml in formic acid: methanol in water (HPLC grade) 60:40. The flow rate was 0.05 ml/min. Solutions of withanolide A, withaferin A and withanolide D were injected at 20, 40, 60, 80,100 ug/ml concentrations of and relationships between peak area and concentrations were plotted. These curves were used to quantify the withanolides in aqueous leaf extracts.

Statistical Analysis

The data of morphological and physiochemical parameters were subjected to One way and Two way ANOVA. Differences among mean treatments were established with Tukey's test and Bonferroni's comparison test.

RESULTS AND DISCUSSION

The methanolic extract had more constituents than other organic extracts. However, in most solvents, the carbohydrates, phenolics, tannins and flavonoids were present. The methanolic extracts of *W. somnífera* leaves did not have terpenoids and saponins. In chloroform and petroleum ether extract, the carbohydrates, proteins, alkaloids, steroids, terpenoids, saponins, steroids and glycosides were absent. The methanolic and distilled water showed presence of maximum number of phytochemicals.

Secondary metabolites (withanolides)

Thin layer chromatography showed the presence of withanolide A, withanolide D and withering A in the leaf extract of *Withania somnífera* (Fig. 7). The R_f values for

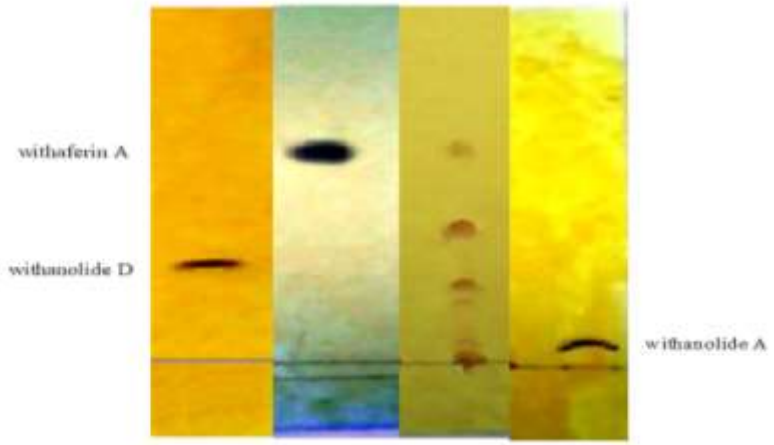


Figure 2. TLC profile of comounds extracted from roots of *Withania somnifera.* Lane A- withanolide D (standard) Rf value 0.19, lane B- withaferin A (standard) with Rf value 0.41, lane D- withanolide A (standard) with Rf value 0.58 and lane C –compounds from the *Withania* extract.

withanolide A, withaferin A and withanolide D were 0 .58, 0.41 and 0.19, respectively. This is shown in lane D of Fig. 2.

(i). IR spectroscopy: Withanolides from leaf extract were eluted from the TLC chromatograms along with standard markers of withanolides and studied using FTIR (Fig. 3). Withanolide A, withanolide D and withaferin A are steroidal lactones and showed similar IR patterns with the presence of a carbonyl group between 1700-1730 wavenumber (cm^{-1}). IR details of withanolide A (standard marker) and purified withanolide A extracted from control plants are given below:

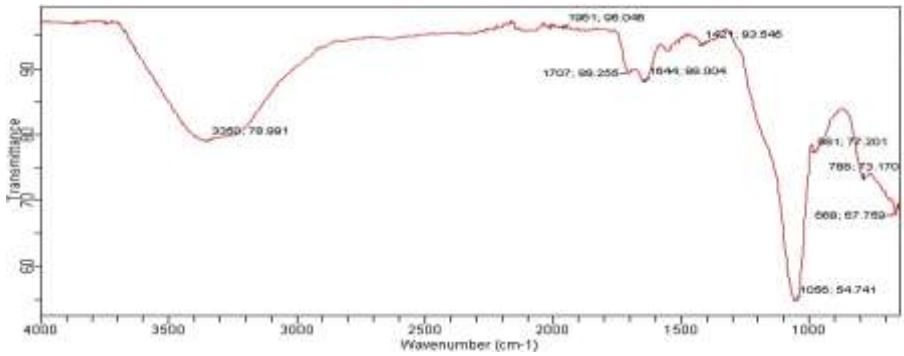


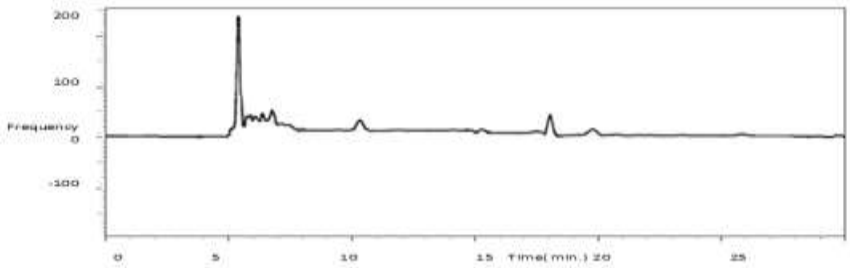
Figure 3. IR - Chromatogram of standard marker withanolide (A) and extracted withanolide (B) from *Withania somnifera*. FTIR graph showing presence of withanolides at wavenumber 1707 (cm^{-1}) (A) and 1719(cm^{-1}) (B).

IR cm^{-1} (Standard withanolide): 3350: (O-H) stretch, 1953: (C-H) stretch, 1707: (C-H) C=O stretch vibration, 1644: (C=O) stretch, 1413: (C-H) asymmetric vibration, 1056: (C-H) deformation.

IR cm^{-1} (withanolide in extract): 3353: (O-H) stretch, 1710: (C-H) C=O stretch vibration,1640: (C=O) stretch, 1413: (C-H) asymmetric vibration, 1056: (C-H) deformation.

The IR values 1707 and 1710 (C=O) clearly indicated the presence of acyl group of lactones. The value at 1056 (C-H) showed the presence of cyclohexane ring. As the peaks were present in both the samples (as shown in Fig.3) data confirmed presence of withanolide.

(ii). HPLC: A typical chromatogram of withanolide A, withaferin A and withanolide D, with optimized condition gave sharp and symmetric peak with specific retention time of 17.743, 10.625 and 20.941, respectively (Fig. 4).



Peaks	Compound	Retention Time
A	Withanolide A	17.800-17.913
B	Withafrein A	10.624-11.046
C	Withanolide D	20.941-21.872

Figure 4. HPLC graph of root extract of withanolide A (WD-A), withaferin A (WF-A) and withanolide D (WD-D).

Figure 5. Effects of foliar spray of *Withania somnifera* L. extracts on growth of *Hordeum vulgare* L. and *Cicer arietinum* and *Cannabis sativa* L. on one month old crops and weed.

Petri Plate Bisoassay (Seed germination)

The *Withania* leaf extracts decreased the growth parameters of *C. sativa* and was most inhibited by pine wood ash as compared to control (Fig. 8). Results showed that *Withania* leaf extract was most inhibitory to stem length of *Cannabis sativa* L. and the inhibiton followed the order: leaf extract (50 %) > wood ash (48 %) > stem extract (24 %) > glyphosate (25 %) > root extract (12 %) over control. In *Hordeum vulgare* stem length followed the order: glyphosate (89 %) > leaf extract (23%) and > wood ash (20 %) than control (Fig 9). Similarly in *Cicer arietinum*, glyphosate caused maximum inhibition in stem length (Fig. 7) followed by leaf extract (88%) and wood ash (22 %) than control (Fig. 10). Results clearly showed that *Withania* was inhibitory to *C. sativa* and was less toxic to crops. Further to prepare an effective herbicidal formulation, eleven combinations were prepared with varied concentration of *Withania* leaf extract, wood ash and distilled water and were tested against studied weeds (Fig. 6). The results showed that in petriplate bioassay extract V (75:25 concentration (*Withania* leaf extract: Wood ash) showed maximum reduction in the seed germination and seedling growth of weed (Fig. 5). Maximum reduction in stem length was 55 % in *Cannabis sativa* L, 20 % in *Hordeum*

vulgare L and 19 % in *Cicer arietinum* L in comparison to control whereas glyphosate decreased the stem length of *Cicer arietinum* L. by 80 % followed by 58 % in *Hordeum vulgare* L and 16 % in *Cannabis* in comparison to control. Extract V showed maximum reduction in root length which was 53 % in *Cannabis* followed by 23 % in *Hordeum vulgare* L and 22 % in *Cicer arietinum* L in comparison to control whereas glyphosate reduced the root length by 75 % in *Cicer*, 73 % in *Hordeum vulgare* L and 18 % in *Cannabis* in comparison to control. *Withania* leaf extract V reduced total dry weight of *Cannabis* by 57 %, 16 % in *Cicer* and 10 % in *Hordeum vulgare* L in comparison to control (Fig. 8). However, the glyphosate reduced the total dry weight by 81 % in *Cicer* followed by 60 % in *Hordeum vulgare* L and 26 % in *Cannabis* as comparison to control plants. Germination is the most important step in the weed establishment (17).

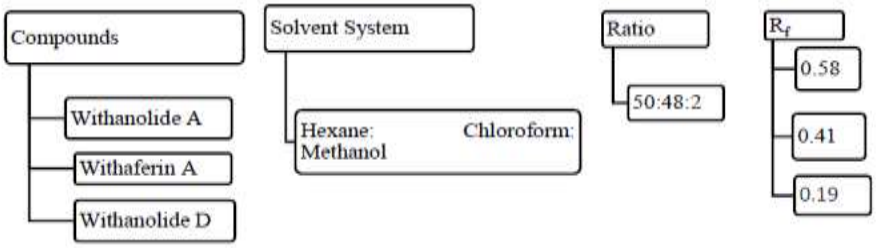


Figure 6. R_f values of withanolide A, withaferin A and withanolide D in *Withania somnifera.*

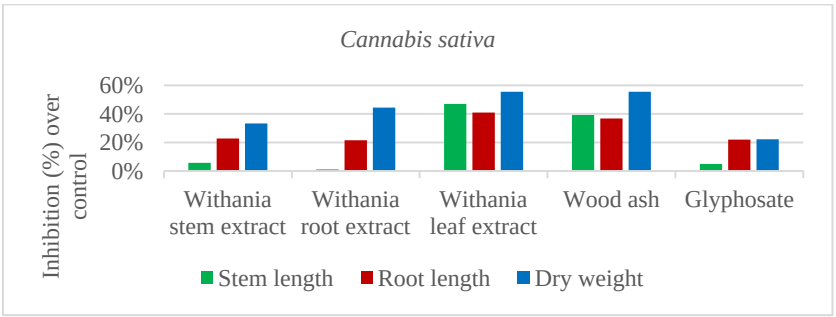


Figure 7. Inhibitory effects of *Withania somnifera* extracts, wood ash and glyphosate on growth of *Cannabis sativa.*

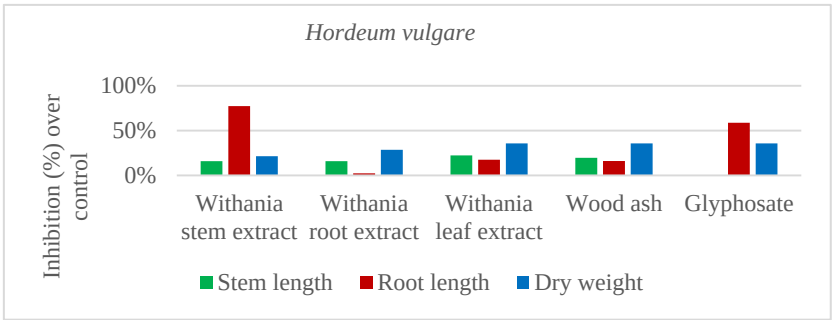


Figure 8. Inhibitory effects of *Withania somnifera* extracts, wood ash and glyphosate on growth of *Hordeum vulgare.*

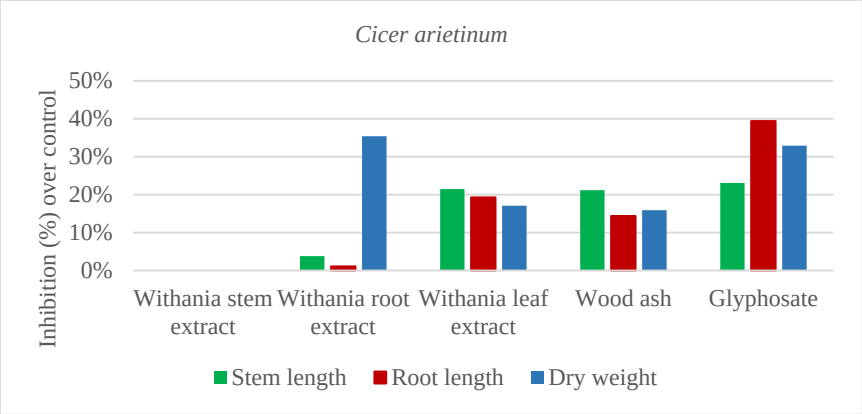


Figure 9. Inhibitory effects of *Withania somnifera* extracts, wood ash and glyphosate on growth of *Cicer arietinum.*

Our results are in agreement with previous work of varios authors. The leaves and stem extracts of *Erythroxylum monogynum* significantly decreased the germination and growth of tomato (2). The *Cassia tora* L exracts inhibited the germination, growth and metabolism of *Parthenium hysterophorus* (11). Seeds germination of *Parthenium* was decreased by aqueous leaf extract of tree species i.e *Azadirachta indica* (L.). A. Juss., *Melia azaduarachta* L. and *Ficus bengalensis* L. (15,18). Herbicidal activity of *Withania somnifera* L.was also reported against the *Parthenium hysterophorus* in Mid Himalayas (19). The results of this study are supported by above findings as *Withania somnifera* L possess variety of allelochemicals in leaves, stem and roots which can have allelopathic effects. The weed seeds germination was plants released allelochemicals (9,10).

Pot Culture (Foliar spray)

Foliar spray bioassay on weed seedlings (Fig. 5) also supported the earlier results. The most effective formulation i.e Formulation V showed maximum reduction in stem length by 57 % as compare to the 18 % in *Hordeum vulgare* and 13 % in *Cicer arietinum* in comparison to control. Root length in *Cannabis* was reduced by 47 % followed by 8 % in *Hordeum vulgare* and 4 % in *Cicer arietinum* in comparison to control. Maximum reduction in fresh weight was observed in *Cannabis sativa* by 62 % followed *Hordeum* by 15 % and *Cicer* by 13 % in comparison to control. *Cannabis sativa* showed maximum reduction in dry weight by 82% followed by 19% in *Hordeum vulgare* and *Cicer arietinum* by 9 % in comparison to control. Maximum decrease in number of leaves was reported 58 % by *Cannabis sativa* followed by 22 % by *Hordeum vulgare* and *Cicer* by 15 % in comparison to control. Number of root branches showed maximum reduction in *Cannabis* by 69% followed by *Hordeum* by 22 % and *Cicer* by 17 % in comparison to control. Maximum reduction in leaf area was showed by 59 % as compare to the 11 % in *Hordeum vulgare* and 4 % in *Cicer arietinum* in comparison to control. Our results are in agreement with other researchers as well.

Allelochemicals affect the plant physiology through multiple functions. They inhibit weed growth, cause stomatal closure, disturb mineral uptake, influence

photosynthesis, affect respiration, induce water stress and damage the enzyme activity, allelochemicals disrupt the H+-ATPase activity in root microsomal membranes and inhibit the growth of targeted weeds due to presence of mitochondrial and tonoplast ATPase inhibitors (18,23). Proper functioning of H+-ATPase enzyme is essential to maintain proton concentration in the rhizosphere, external to root cell membrane. Inhibition of this enzyme retards the water uptake capacity of plant (7) This produces an electrochemical gradient to drive the water uptake. Oxidative suppress and reactive oxygen species (ROS) generations causes transformations in physiological and biochemical process. This resulted in an increase in malondialdehyde contents, ascorbate peroxidases and guaiacol peroxidases that ultimately retard the growth of weeds (25). The formulated herbicidal formulation has shown a very little effect on the studied crops i.e *Hordeum vulgare* Land *Cicer arietinum.*

Similar results have also been reported by many researchers. Root, stem and leaf aqueous extracts of *Eclipta alba* L were applied to determine their effect on seed germination and seedling growth (26,30). The aqueous extracts from root, stem and leaf had no effect on seed germination of *Phaseolus aureus* and *Oryza sativa.* Likewise, allelopathic and bioherbicidal potential of *Cladonia verticillaris* on the germination and growth of *Lactuca sativa* (27,29). The possible reasons could be herbicidal plant extract resistance in crop plants is usually conferred by mechanisms. First, resistance can be due to lack of herbicide activity at the site of action target site not present, target site present but herbicide binding site absent or altered, excessive amount of the target enzyme relative to the amount of herbicide. A non-target site mutation such as rapid metabolic detoxification of the herbicide is another mechanism that can allow plants to survive with little or no irreversible effects from the herbicide applications (28, 31). As medicinal plants like *Withania somnifera* is storehouse of phytochemicals and is herbicidal to weeds, there is an opportunity to commercialize its extracts as bioherbicide, as its applications are safe, cheap and effective for the weed management in Himalayan region.

CONCLUSIONS

The *Withania* leaf extract and wood ash formulation [(75:25 concentration (Withania leaf extract: wood ash)] controlled the *C. sativa* weed and was harmless to crops. The herbicidal property of *Withania somnifera* could be used to control this weed. The formulation effectively inhibited the germination and growth of *C. sativa* weed and had very little harmful effects on germination and growth of crops. This formulation may help in developing effective method to eradicate this weed from Himalaya. Its chemical components inhibitory to germination and growth of *C. sativa* needs to be developed as ecofriendly herbicide.

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REFERENCES

1. Abou Zid, S.F., Bassuony, E.l. and Choudhary, M.I. (2010). Withaferin A production by root cultures of *Withania coagulans* L. *International Journal of Applied Research in Natural Products* **3**: 23-27.
2. Ahuja, N., Singh, H.P., Batish, D.R. and Kohli, R.K., (2015). Eugenol-inhibited root growth in *Avena fatua* involves ROS-mediated oxidative damage. *Pesticide Biochemistry and Physiology* **118(1)**:64-70.
3. Alagesaboopathi, C. (2014). Allelopathic effect of aqueous extract of *Erythroxylum monogynum* Roxb. on germination and growth of *Solanum lycopersicum* Mill. var. PKM-1. *International Journal of Scientific Research* **3(8)**:1091-1094.
4. Ashraf, R., Sultana, B., Yaqoob, S. and Iqbal, M. (2017). Allelochemicals and crop management, A review. *Current Science Perspectives*. **3(1)**:1-13.
5. Bani, S., Gautum, M. and Khan, F.A. (2006). Selective Th1 up regulation activity of *Withania somnifera* aqueous extract in an experimental system using flow cytometry. *Journal of Ethno pharmacology* **107**: 107-115.
6. Bouhaouel, A. Gfeller, Kh. Boudabous, M.L. Fauconnier, O. Slama AyedH. Slim Amara and P. Du Jardin (2020). Effects of physico-chemical and biological properties of soil on the allelopathic activity of barley (*Hordeum vulgare* L. subsp. vulgare) root exudates against *Bromus diandrus* Roth. and *Stelleria media* L. weeds. *Allelopathy Journal* **49 (1)**: 17-34.
7. Chowdhary, H.J. (1991). Himachal Pradesh. In: *Floristic Diversity and Conservation Stratergies in india* (Eds., V. Mudgal and P.K. Hajra). Bottanical survey of India, Calcutta. Volume **1(1)**:845-887.
8. Delye, C., Jasieniuk, M. and Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. *Trends Genetics* **29**: 649-658.
9. Fickett, N.D., Boerboom, C.M. and Stoltenberg, D.E. (2013). Predicted corn yield loss due to weed competition prior to postemergence herbicide application on Wisconsin farms. *Weed Technology* **27(1)**: 54-62.
10. Bradley, D.H., Albert, J.F., Alan, M., Marie, J., Anil, S. and Amit J.J. (2014). Herbicide resistant weeds and crops. *Principles of Weed Control* **4(1):** 168-188.
11. Hollander, N.G., Bastiaans, L. and Kropff, M.J. (2007). Clover as a cover crop for weed suppression in an intercropping design. Characteristics of several clover species. *European Journal of Agronomy* **26(2):** 92-103.
12. Hozawa, M. and Nawata, E. (2020). Allelopathia effects of leaf litter leachates of *Ulex europaeus* L on other species and its own seed germination. *Allelopathy Journal* **9:** 217-218.
13. Holm, L.G., Plucknett, D.L., Pancho, J.V. and Herberger, J.P. (1977). *Cirsium arvense* (L.) Scop. The World's Worst Weed–Distribution and Biology. East-West Centre, University Press of Hawaii, Honolulu. **1(1):** 217-224.
14. Kumar, N. (2006). Medicinal plants, future source of pesticides. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences.* **8(1):** 57-59.
15. Lee, S.O., Choi, G.J., Jang, K.S., Lim, H.K., Cho, K.Y. and Kim, J.C. (2007). Antifungal activity of five plant essential oils as fumigant against postharvest and soil borne plant pathogenic fungi. *The Plant Pathology Journal* **23(2):**97-102.
16. Mallory-Smith, C.A. and Retzinger, E.J. (2003). Revised classification of herbicides by site of action for weed resistance management strategies. *Weed Technology* **17**: 605-619.
17. Misra, L.N., Lal, P., Sangwan, R.S., Sangwan, N.S., Uniyal, G.C. and Tuli, R. (2005). Unusually sulfated and oxygenated steroids from *Withania somnifera*. *Phytochemistry* **66**: 170-178
18. Mukhopadhyay, A. Singh, A. Somvanshil, and V.S. Patanjali, N. (2020). Novel bioinsecticidal gel formulation with improved shelf life and infectivity. *Allelopathy Journal* **49:** 257-270.
19. Mei-Ren Pan, Hui-Chiu Chang, Yang-Chang Wu, Chao-Cheng Huang, and Wen-Chun Hung (2009). Tubocapsanolide A inhibits transforming growth factor to suppress NF kappaB-induced CCR7. *Biological Chemistry* **285:** 2746-54.
20. Todkar, S., Chavan, V. and Kulkarni, A.S. (2010). Screening of secondary metabolite and antibacterial Activity of *Acacia concinna* L. *Research Journal of Microbiology* **5**: 974-979.
21. Prasad, P.V., Boote, K.J. Allen, J.L.H., Sheehy, J.E. and Thomas, J.M.G. (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crop Research* **95**: 398-411.
22. Rial-Otero R, Arias-Estévez M, López-Periago E, Cancho-Grande B, Simal-Gándara J. (2005). Variation in concentrations of the fungicides tebuconazole and dichlofluanid following successive applications to greenhouse-grown lettuces. *Journal of Agricultural and Food chemistry* **53:** 4471-5

23. Rohini, V.K. and Sankara Rao, K. (2000). Embryo transformation, a practical approach for realizing transgenic plants of safflower (*Carthamus tinctorius* L.). *Annals of Botany* **86**: 1043-1049.
24. Sekar, K.C. (2011). Invasive alien plants of Indian Himalayan region, diversity and implication. *American Journal of Plant Sciences* **3(2)**:177-187.
25. Sharma M, Puri S. Effect of temperature and light stress on the morphology physiology and secondary metabolites of *Withania somnifera* (L.) Dunal and its potential as herbicide on the Himalayan weeds. Shodhganga, 2016: 54-74.
26. Sharma, M. and Puri, S. (2017). Temperature stress mediated oxidative and antioxidant defense in *Withania somnifera* Dunal. *Journal of Chemical and Pharmaceutical Sciences* **10**: 892-902.
27. Tao, B. and Hu, F. (2009). *Practical Technology of Chemical Weed control*. Chemical Industry Press, Beijing.pp 13-18.
28. Vaghasiya, Y., Dave, R. and Chanda, S. (2011). Phytochemical analysis of some medicinal plants from western region of India. *Research Journal of Medicinal Plants* **5**: 567-576.
29. Victor, N.O. and Chidi, O. (2009). Phytochemical constituents of some selected medicinal plants. *African Journal of Pure and Applied Chemistry* **3**: 228-233.
30. Wakabayashi, K. and Boger, P. (2002). Target sites for herbicides: Entering the 21st century. *Pest Management Science* **58**: 1149-1154.
31. Zhang, C.X., Chao, X., Ni, H.W., Wei, S.H., Huang, H.J., Liu, Y., Cui, H.L., Sui, B.F., Zhang, M. and Guo, F. (2009). Current advances in research on herbicide resistance. *Scientia Agricultura Sinica* **42**: 1274-1288. (Chinese).