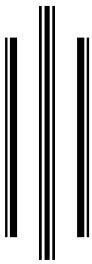
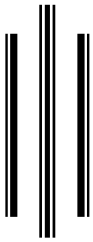
Allelopathic influences of *Artemisia dubia* Wall. Ex. Besser on seed germination of *Parthenium hysterophorus* L.



A Dissertation
Submitted for the Partial Fulfillment of the
Requirement of M. Sc. in Botany



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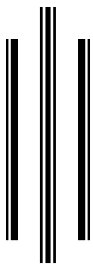

Central Department of Botany
Tribhuvan University, Kirtipur
Kathmandu, Nepal
2008

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RECOMMENDATION

This is to certify that dissertation work entitled "Allelopathic Influences of *Artemisia dubia* Wall. Ex. Besser on Seed Germination of *Parthenium hysterophorus* L." has been carried out by Ms. Manisha Sharma for the partial fulfillment of M. Sc. Degree in Botany, under my supervision. The entire work is based on the results of her own work and has not been submitted for any other degree to the best of my knowledge.

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LETTER OF APPROVAL

This dissertation paper submitted by Ms. Manisha Sharma entitled "Allelopathic Influences of *Artemisia dubia* Wall. Ex. Besser on Seed Germination of *Parthenium hysterophorus* L." has been accepted as a partial fulfillment of Masters of Science in Botany.

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Manisha Sharma

ABBREVIATIONS

cm	Centimeter
DMSO	Dimethyl sulphoxide
G	Gram
h	Hour
L	Liter
m	Meter
mg	Milligram
ml	Milliliter
mm	Millimeter
nl	Nanoliter
ppm	Parts per million
S.D.	Standard Deviation
v	Volume
w	Weight
µ	Micro

ABSTRACT

Screening plant produced phytotoxins (allelochemicals) through bioassay is an accepted strategic tool employed for the discovery of potential pesticides for weed management. The present laboratory based study was undertaken to serve the purpose of preliminary screening of phytotoxicity of *Artemisia dubia* against an invasive composite *Parthenium hysterophorus*.

Allelopathic effect of aqueous (leachate and decomposed) extract, solvent extract (hexane, chloroform, methanol and water), soil amended extract with leaf, stem and root and essential oil of donor plant *Artemisia dubia* was studied on germination and seedling vigour of *Parthenium hysterophorus*. Extraction and quantification of different parts in different solvent was done by percolation method while hydro distillation method was followed for essential oil extraction. Bioassay was done by allowing *P. hysterophorus* seed to germinate in petri dishes (for solvent, aqueous and essential oil) and disposable plastic plates (for plant parts amended soil) in laboratory condition against varied concentrations of prepared extracts of *Artemisia dubia.*

Yield of crude extract (solvent and aqueous) was high for leaf compared to stem and root. All the tested extract (aqueous, solvent, essential oil and soil amended with plant parts) from different plant parts significantly checked the germination of *Parthenium hysterophorus* in higher concentration with the effect being more pronounced due to leaf of *Artemisia dubia.* Linear growth of root and shoot also followed the same pattern. The order of allelopathic influence was leaf > stem > root in all the cases and it was concentration dependent. Length of root was retarded while stem length experienced no effect, sometime elongation (stem and root extract of leachate, stem extract of hexane and chloroform) and sometime retardation (aqueous decomposed extract of leaf, leaf amended soil and essential oil). Aqueous decomposed leaf extract, soil amended with leaf parts and essential oil were found to be most inhibitory.

These results provide ample evidence that allelopathic potential exist in *Artemisia dubia* and this can be exploited for the control of *Parthenium hysterophorus.*

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1. INTRODUCTION

1.1 Background

With confrontation to environment; inclusive of physical, chemical and biological factors, every organism evolves species idiosyncratic specificity to counteract/interact for own survival and success. Interactive response is more peculiar in plant's world, in addition to usual physical, biological cooperative/competitive interactions, as a compensation to sedentary life forms plants have evolved chemical interactions that is mediated via bio-active substances, the secondary metabolites produced as offshoot from the primary metabolism process which play important role in ecosystem as signal, recognition, defense substances, inhibitors and poisons rather than being utilized as nutrients. And this chemical mediated interaction determines the sociability of plant and its success in nature. Secondary metabolites mediated interactions is widely accepted in ecological perspective where these determine plant-herbivore interactions (Rosenthal and Berenbaum, 1991), regulate soil biotic interactions (Wardle & Lavelle, 1997), determine processes associated with nutrient cycling (Northup *et al*., 1995), decomposition (Horner *et al*., 1988) and may also work in shaping plant community (Wardle *et al*., 1998). These chemical communicational interrelationships are however very obscure and complex in nature such that effects of bioactive substance can't be strictly classified and thus the science for defining chemo-ecological relationship remains in infancy till date.

1.2 Allelopathy

Allelopathy is an interference mechanism mediated by chemical products of plants released to environment in which there is exertion of effect (usually negative) on associated plants. Though been noted anciently by farmers and gardeners, the earlier recorded reorganization dates back to 300 BC and it was by Theophrastus. The means was termed allelopathy (Greek words, *allelon* = mutual and *pathos* =harm) by Molish in 1937 to refer to reciprocal suffering of two organisms. Rice (1984) reinforced this definition in the first monograph on allelopathy, defining it as "any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through the production of chemicals that escape into the environment" (Rizvi and Rizvi, 1992).

It is plant/plant biochemical interactions that may be harmful or beneficial; it involves release of chemical by plant in the environment in the manner that imposes effect on other plants sharing the same habitat. This has been recognized at all level of complexity, from microorganism to higher plants, and is inextricably interwoven into ecological phenomena. In this context, competition for food and living space is often carried out chemically; all sorts of antibiotics, toxins, germination and growth inhibitors or stimulants may be released for these purposes. Adsorbed by the surrounding soil or upon direct action through the air, chemicals are used by plants and microorganisms to manipulate partners, competitors, and ecosystems.

Chemicals that impose allelopathic influences are called allelochemicals and are classified as secondary plant metabolites which have apparently nothing to do with elemental plant's survival process connected to primary metabolism. Not again all of secondary metabolites are allelochemicals, within wide array of tens of thousands of these compounds produced; only a limited number has been implicated to allelochemicals (Rice, 1984). Allelochemicals have been classified basically to the groups of chemicals namely phenolics, terpenoids, alkaloids, steroids and quinones (Einhelling and Leather, 1988). These are present virtually in all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollen. Production of allelochemicals is under influence of genetical and environmental factors. They may be released from plants into the environment by means of four ecological processes; volatilization, leaching, root exudation, and decomposition of plant residues (Putnam, 1985). Several chemicals can be released together and may exert toxicities in an additive or synergistic manner. The concentration of a single substance in field situations is generally below its inhihibitory threshold. Allelopathic interferences often result from the joint action of several different compounds. Again, biological activities of receiver plants to allelochemicals are known to be concentration dependent with a response threshold. Responses are characteristically, stimulation at low concentrations of allelochemicals, and inhibition as the concentration increases (Lovett, 1989).

This mechanism has been widely observed from gross morphological level to the biochemical level. The visible response that has been noted in plants exposed to allelopathic chemicals are retardation in growth and development like inhibition of germination rate, seeds darkening and swelling, reduction of root or radicle and shoot or coleoptiles extension**,** swelling or necrosis of root tips, curling of root axis, discoloration, lack of root hairs, increased number of seminal roots, reduced dry weight accumulation, and lowered reproductive efficiency.

These observations have necessitated the better understanding of allelopathy and opened a new way to the scientific world whose interest is in the field where plant interactions are critical. Incorporating allelopathy into weed management has been a new area which has lifted this branch of science to the practical application and this has drawn the attention of scientist today as a tool for the discovery of novel compounds which may also exhibit herbicidal activity and consequently work as natural herbicides or as a template for development of synthetic herbicides which could be more environmentally safe. Today allelopathy is taken as an important strategic tool of chemical ecology approach that is employed for the recognition and extraction of natural products generally the phytotoxins for utility as natural pesticides.

1.3 Statement of problem

Weeds are naturally selected opportunistic genotypes of species that have evolved a number of adaptive attributes like abundant seed production, dispersal, varied seed dormancies, high competitive potentiality and vegetative propagation that make them thrive well and even shape themselves with the changed situation in the environment thus they claim a substantive share of nutrient and space (competition, allelopathy). Even with a miniscule share of approximately 1% in world's flora (Qasem and Foy, 2001), weeds are known to cause harm to crops and other plants. Again, weeds sometimes enter the sectors of human affairs as health, food security and economy. This makes it imperative to control them and as a result number of weed management practices has been available today.

Parthenium hysterophorus L. locally called "Kanike ghas" is a new invasive and exotic weed species that has been reported to be rapidly spreading in Nepal. It is of a recent introduction (Hara *et al*., 1982; Mishra, 1991) to this land but it has high amplitude of distribution along east west gradient in the tropics and is on its way towards subtropics (Adhikari and Tiwari, 2004). Owing to its prolific seed production (Navie *et al.*, 1996), germination capability in wide amplitude of climatic condition (Tamado *et al.*, 2002 a) and allelopathic potentiality (Tefera, 2002) it stands as aggressive colonizer. *P. hysterophorus* is known for its serious human health risks (Lonkar *et al.*, 1974; McFadyen, 1995). Hay fever, asthma, allergies and parthenium dermatitis are major health hazards in human caused by dust, debris and pollen of the plant. Again, it's impact on crop yield (Khosola and Sobti, 1981; Tamado *et al*., 2002 b), forage production in grass land (Nath, 1988), on animal husbandry (Kadhane *et al*., 1992) is a

well documented fact that is recognized world wide. Lethality due to this plant does not end here; it is suspected to be host for several crop pathogens (Ovies and Larrinaga, 1988; Goswami, 2007). In Nepal, intensive studies on documentation of its impacts on health, crops or biodiversity is still lacking and inventory and assessment of invasive species in Nepal done by The World Conservation Union (IUCN) has ranked it to be of moderate threat in terms of invasiveness. However, it never means that it is of least importance. In light of global acceptance of its noxious character and initiatives to control it and exhaustive invasive potentiality recognized world wide, one can realize necessity to control it before it actually creeps to wreak havoc.

Weed control is heavily dependent on the synthetic chemical formulations (herbicides) and this has been the basis for high input commercial agriculture ensuring high production. However, its costs on people's health and environment, evolving herbicide-resistant ecotypes and cross-resistance in weeds has made scientists to look for environment friendly, safe and sustainable alternatives. This has urged the shift in direction of weed control to weed management rather than the conventional weed removal that exclusively uses the synthetic herbicides. Natural products (allelochemicals) may give way to discovery of new herbicides which are more environmentally benign. Understanding factors controlling their production and release, affecting their activation and inactivation, nature and mechanism of action facilitates possible exploitation of natural chemicals in a beneficial way such that it forwards preserving and improving crop yield and quality. Thus knowledge about the allelochemical behavior of the plant is of obvious importance to the environment friendly weed management.

1.4 Scope of study

It will be fallacious to look only at the negative side of weed; economically weed is also defined as the plant whose virtues have not been assessed fully. Weed has also been noted as facilitator for the other plant species at its low density in the environment. Best way of managing weed is to find its usefulness and make economic use of it, to promote it to level of wanted plant and its selective chemical interference to control unwanted and noxious weeds, as a stimulant to crop fields and biological pesticides; and the knowledge of chemistry and biology of allelochemicals provides many opportunities for such practical application. So, the science of allelopathy has received a great concern for researches, and allelopathic aspects of

many weeds are being investigated considerably, however work in this sector is very scant in Nepal though it harbors a wide range of allelopathic plants.

Artmesia dubia Wall. Ex. Besser is also a problematic weed in Nepal found from eastern to western zone, in the altitudinal range of 1200-3400 m (Press *et al*., 2000). It has a vigorous growth, is very competititve and thus pure stand of *Artemisia* often can be seen in open places. This plant dramatically reduces biodiversity and infestation to crop field also reduces crop quality and it has much higher maintenance expense in turf land and other urban setting. *Artemisia* produces different flavonoids, phenolics, terpenoids and wide array other secondary products with a higher concentration in aerial portions which as allelochemicals influences other ecosystem components, that in turn drive interaction which determine the community structure. *A. dubia* as other members of its genus may have more ranging effects than simply altering the success of other plants. On a beneficial side, *Artemisia* is known for potential insecticidal, repellant (Abdelgaleil *et al*., 2007; Wang *et al*., 2006), nematicidal (Al-Banna *et al*., 2003), antifungal (Culini *et al*., 2006; Meepagala *et al*., 2003), medicinal (Seo *et al*., 2001; Mueller *et al*., 2000), and herbicidal (Modallal and Al-Charchafchi, 2006) properties which secures the position of *Artemisia* spp. as store house of chemicals with diverse biological activities.

Use of easily available and viable local resource that is already in traditional practice by farmer in a form of botanical pesticides for pest control measures are still finding importance. Therefore, allelopathy has been a growing new branch of science which is being utilized for pest control. The following research conducted to screen phytotoxic potentiality of. *Artemisia dubia* against *Parthenium hysterophorus* as a source to natural products for its control holds the scope of finding natural solution to weed management.

1.5 Description of the test plants

1.5.1 Donor plant (*Artemisia dubia* Wall. Ex. Besser)

1.5.1.1 Classification

Class-Dicotyledons

Sub-class-Gamopetalae

Series-Bicarpellatae

Order-Asterales

Family- Asteraceae

Genus-*Artemisia*

Species- *A. dubia*

Common name-Mugwort

Nepali name-Titepati

1.5.1.2 Ecology/Taxonomy

Artemisia plant most probably seems to have originated in the mountainous regions of north-western Asia (Wang, 2004). These are found growing wild and abundantly all over the temperate and cold temperate zones of the world. Grow well in dry areas of fallow land and along road sides and also in wet habitats. The plant parts are fragrant and produce allelopathic substances.

Tall perennial herb, stem pubescent, lower leaves petioled, ovate with lobe entire, green and glabrescent above, grey/whitish tomentose beneath, upper leaves 3-fid, or entire, lanceolate, head sessile, panicles raceme, involucre sub-globose 1.5-2 mm diameter, phyllaris ovate or oblong c. 2 × 1 mm, outer one sparsely pilose at base outmost smaller, female flower 8-11, corollas 1 mm, central flower male 5-11, corollas 2 mm, ovaries ± absent, achenes small, smooth, brown, pappus absent (Malla *et al*., 1986).

1.5.2 Receptor plant (*Parthenium hysterophorus* L.)

1.5.2.1 Classification

Class-Dicotyledons

Sub-class-Gamopetalae

Series-Bicarpellatae

Order-Asterales

Family-Asteraceae

Genus-*Parthenium*

Species- *P. hysterophorus*

Common name-Ragweed

Nepali name- Kanike ghans, padke phul

1.5.2.2 Ecology/Taxonomy

Parthenium plant native to tropical and subtropical America grows well in fallow lands and roadside in tropical and temperate regions and is capable to colonize most soil types. This is reported to pose inhibition on surrounding vegetation. Provided with good rain fall and warm temperature it can grow at any time of year (Adhikari and Tiwari, 2004).

Annual, erect and profusely branched herb. Height varies between 50-150 cm, stem highly branched leaf with profusely dissected leaflets; flower heads occur on a corymb, phyllaries 10 in 2 series, ovate, dull white, 3-4 mm in diameter; disc floret: numerous, dull white; stamen - 4, anther- exerted; ovary sterile; ray floret: found just opposite to inner phyllaries, only 5 ray florets per flower head, corolla obsolete, stamen-absent, stigma-parted, style short, ovary oval, dorsiventrally flattened; fruit cypsela, each flower head bearing 5 cypsela, flat and triangular in shape with thin, white, spoon shaped appendages (Maharjan, 2006).

1.6 Objectives of the study

Present study was undertaken to look at the phytotoxic potentiality of *Artemisia dubia* (donor plant) against *Parthenium hysterophorus* (receptor plant). However, research accounts following specific objectives:-

- To find the effect of decompose, leachate and solvent extract of different parts of *A. dubia* on germination and seedling vigour of test seeds of *P. hysterophorus*
- To find the effect of essential oils of *A. dubia* on germination of *P. hysterophorus.*
- To find the effect of soil amended with different plant parts of *A. dubia* on germination and seedling vigour of *P. hysterophorus*.

2. REVIEW OF LITERATURE

2.1 Weed status of *Parthenium hysterophorus* (ragweed)

Narasimhan *et al*. (1977) conducted an experiment to see the effect of *Parthenium hysterophorus* in cattle. It was found that *P. hysterophorus* when fed to buffalo bull calves and cross bred bull calves resulted in acute toxicity leading to death. The former animals developed severe dermatitis. Autospy revealed ulceration of alimentary tract. Extensive pathological changes were noticed in liver, kidney and skin.

Chippendale and Panetta (1994**)** evaluated the economic cost of *Parthenium hysterophorus* in Central Queensland on cattle industry with help of mail survey. It was found that annual loss due to this plant was in vicinity of $16.5million and losses comprised opportunity costs (e.g. reduced stock numbers and live weight gains) as well as additional production and control costs.

Kololgi *et al*. (1997) on diagonostic case studies found that itching had been main symptoms to contact dermatitis; patients who developed itchy and oozing lesions were diagnosed to suffer contact irritant dermatitis and oedema had been the symptom to phyto photodermatitis. All these dermatological hazards were found to be due to *Parthenium hysterophorus.*

Lemessa (2001) on a field assessment mission of UN-emergencies Unit Ehiopia for analysis of food security situation in East and West Hararghe Zones of Oromia Region found that the lowland crops along with moisture stress are suffering *Parthenium hysterophorus* impacts. It was reported that weed is spreading and invading both crop fields and grazing areas at an alarming pace. Despite the prevailing moisture stress it was flourishing and giving hard time to farmers by competing with crops, reducing yields and causing bad taste of milk and honey when animal ate the weed and bees collected nectars from the plant.

Tamado *et al.* (2002 b) studied the interference of *Parthenium hysterophorus* density and duration of competition on grain yield of sorghum at lowland and intermediate altitude sites in Ethiopia. They found that yield loss was severely affected by *P. hysterophorus* weed density, peaking up to 97%, even at the low weed density of three plants per metre induced yield loss of 69%.

Annapurana and Singh (2003) evaluated the performance of *Parthenium hysterophorus* with respect to soil quality on different attributes as phenology, growth variables and seed traits. It was found that high clay content in soils prolonged rosette stage, enhance growth rate in height and diameter and hampered root growth, but promoted biomass allocation to shoots. Seed mass declined whereas seed production increased in coarser soils in contrast to larger seeds in soils rich in clay content. This variation in fundamental functional traits suggests that *P. hysterophsorus* can adjust to variety of habitat conditions.

Navie *et al*. (2004) determined the germinable soil seed bank at two sites in Queensland infested by *Parthenium hysterophorus* at different times and found them to be dominated by *P. hysterophorus* with the heavily infested site with higher value. However, it was depended on season. Species richness, diversity of seed bank, and abundance of many species was lower during spring (when the dense infestations of the weed originate). The domination of seedbank of *P. hysterophorus* shows it is having substantial negative impact on ecology of plant community. Again diversity of seed bank was lower than grassland without it representing prolonged presence of *P. hysterophorus* may have reduced the seed banks of other species thus implicating to loss of regeneration of some of native species.

Batish *et al*. (2005) conducted a study to determine *Parthenium hysterophorus* residue possible interaction with soil nutrient status. The extracts from 1, 2 and 4 weeks old residue significantly inhibited the seedling growth of two test crops-radish and mustard but phytotoxicity decreased with increasing age of residue. When the nutrients of soil amended with residue were tested for N, P, K and micronutrient it was found to increase compared to non amended soils thus ruling out any possibility of their direct involvement in observed growth inhibitions. However residue extracts and amended soils were observed to be rich in phenolics- known allelochemicals.

Maharjan *et al*. (2007) studied allelopathic effects of aqueous extracts of *Parthenium hysterophorus* on seed germination and seedling growth of three cereal crops, three cultivated crucifers and two wild asteraceae species. It was found that seed germination of all crucifer species was completely inhibited at >2% concentration of leaf extract while in *Triticum aestivum* L. and *Ageritina adenophora* (Spreng) King and HE Robins it was at > 6% concentration. In other species except *Zea mays* L., complete inhibition of germination was

recorded only at 10 % concentration. Again strong inhibition was noted in root elongation of seedling in cereals and shoot elongation in crucifers and Arteraceae species.

2.2 Phytotoxicity of *Artemisia* spp.

Yun and Kil (1992) assessed phytotoxic effect of residues of *Artemisia princeps* var. *orientalies* (Pampan.) Hara on various plants. Various field and laboratory studies were conducted. It was found that seedling elongation and dry weight of receptor plants were inversely proportional to the concentration and incubation time of dry leaves of *A. princeps* var.*orientalis* in vermiculite. In seedling growth tests with abandoned field soils and soil underneath wormwood, elongation, dry weight and caloric content of seedlings grown in the soil from under wormwood plants were found severely inhibited.

Bagchi *et al.* (1997) tested effect of arteether, a derivative of artemisinin from *Artemisia annua* on seedling growth of dicotyledons and monocotyledons and compared its toxicity with the derivatives of *A. annua* as artemisinic acid, arteannum B and artemisin. Arteether was found to be most effective growth inhibitor among tested compounds and its inhibitory effect was more pronounced on root than on shoot. Again artemisin, precursor of arteether was observed more toxic to monocotyledons and less toxic to dicotyledonous weed in comparison to arteether.

Lydon *et al.* (1997) evaluated the effect of leaf tissue extracts from *Artemisia annua* L. and pure artemisin on plant growth when incorporated into sandy loam soil. Extract of dried leaf tissue on methylene chloride, ethanol and water was incorporated in soil at rates equivalent to 0, 0.3, 0.73 or 1.1% (w/w) based on soil dry weight. Peatmoss treated with extracts (artemisin) was incorporated in to soil at a rate equivalent to the 0.73 %(w/w) treatments. They observed that annual wormwood leaf tissue and methyl chloride extract treatments were the only treatment that resulted in a reduction in seedling survival. Furthermore they predicted that the aqueous extract, which did not contain artemisin, and the extract residue had activities similar to that of the artemisin treatment, that's why they concluded that the allelopathic effect of annual wormwood cannot be attributed to artemisin.

Yun and Maun (1997) conducted a green house experiment to test allelopathic effect of *Artemisia campestris* sub sp. *caudata* (Michx.) Hall and Clements on seed germination as

well as seedling growth of several sand dune species and colonization by microrhizal fungi. Aqueous extract of *A. campestris* sub sp. *caudata* showed no inhibitory effect on seed germination, seedling elongation or dry weight growth of plants at lower concentrations (10 and 50%) but 100% concentration of the extracts caused varying degrees of inhibition of *Elymus canadensis* L. seedlings. The percentage germination of test species in soil from the rhizophere of *A. campestris* sub sp. *caudata* was significantly lower than that of control. The leaf area and dry weight were also lower but the differences were not significant.

Inderjit and Foy (1999) examined the effect of soil amended with *Artemisia vulagris* L. leaf material on seedling growth of *Trifolium repens* L.. Addition of *A. vulgaris* leachate to soil resulted in chemical change in the soil including changes in available water-soluble phenolic and suppressed red clover seedling growth when compared to that in non-amended soils. They suggested that mugwort allelochemicals play a role in interference with red clover growth.

Rao *et al*. (1999) studied effect of *Artemisia annua* oil on development and reproduction of *Dysdercus koenigii* F. (Hem., Pyrrchocoridae). It was found, mortality was dose dependent with highest (100%) being at 1µml/nymph. Treatment decreased haemolymph protein concentration. Adults which emerged from treated nymph showed poor ovary development and also there was greater median neurosecretory activity in treated insects compared with control insects till day 6 of adult life.

Escudero *et al*. (2000) evaluated allelopathic role of *Artemisia herba-alba* Asso to explain community pattern of Central Spain showing sharp ecotone between a gypsophile sparse community dominated by *Helianthus squamatum* (L.) Dum Cours. and nitrophilous community on gypsum alluvial soil dominated by *A. herb-alba*. Results confirmed inhibitory effect of aqueous extract on final germination of seeds of *H. squamatum.* Assay with soil of *Artemisia* community also suggested the same fact. It was thus concluded that spatial pattern detected could be at least partially controlled by interference through allelopathy which assisted in exclusion of *H. squamatum* plant from alluvial soil.

Preston *et al*. (2002), with a combination of field and laboratory studies examined effect of Methyl jasmonate (MeJA), a biologically active compound released by sagebrush(*Artemisia* sp.) plant by both air and water transport on seed germination of *Nicotiana attenuata* (S.Watson) Torr.. Sagebrush was found to interact allelopathically with seed bank of *N.*

attenuata in field experiments. In laboratory experiment it was found that exposure of seeds to sagebrush emissions resulted in germination delay again exposure to volatile and aqueous MeJA also inhibited germination of *N. attenuata* seeds at that quantities that are released naturally from sagebrush. Again proportion of germination inhibition due to MeJA was calculated and it was found that 16-60% of inhibitory activity of original sagebrush extract could be attributed MeJA.

Cho *et al*. (2003) isolated growth inhibitors seco-tanapatholides A and B (sesquiterpene lactones) from *Artemisia priceps* var. *orientalis* and tested them agaist human intestinal bacteria using impregnated paper disc method. It was found to inhibit *Clostridium perfingins, Bacteroides gragilis* and *Staphylococcus aureus* however tested compounds did not affect test lactic acid producing bacteria (*Bifidobacterium adolescentis*, *Bif. breve*, *Lacto bacillus acidophilus* and *Lact. casei*) and *Escherichia coli*.

Poudel (2004) performed an experiment to assess allelopathic effect of *Artemisia dubia* on seedling of rice and barnyard grass. Study was focused on the allelopathic effect of aqueous (decomposed) extract, leachate and solvent ectract (hexane, methanol, and aqueous) of leaf, stem and root of plant on seeds of rice and barnyard grass germination. He also studied effect of mulch in seedling elongation of test seeds and identified main group of chemical constituent of *A. dubia*. The result showed that the allelopathic effect of all treatments were significantly inhibitory to germination of barnyard grass but no effect on germination of rice seeds. Leaf mulch significantly retarded the growth of barnyard grass as compared to rice. He suggested that the inhibitory effect of leaf mulch to seedling growth of barnyard grass to be a principal to check barnyardgrass infestation in rice seedbed and field.

Parajuli *et al.* (2005) studied efficacy of essential oils of *Artemisia dubia, A. gmelinii* (Stechman) Webb and other plants against *Alternaria brassicola*. Fungitoxicoty was assessed by poison food technique and percentage growth inhibition in different concentration was calculated. Mycelial inhibition on test fungi was noted. It was found that maximum inhibition of 64.44 % was observed at 10,000 ppm concentration of oil for *A. dubia* and 33.33% for *A. gmelinii.*

Karban (2007) conducted a series of lath house and field experiments by clipping foliar part of *Artemisia tridentata* Nutt. to find if there is link between plant damage and germination

inhibition of near by seeds. He found that germination of native forbs and grasses were reduced in associated with clipped compared with unclipped sagebrush plant and air contact was required for this germination inhibition.

Rai and Sakya (2007) studied cytological effect of *Artemisia vulgaris* L. in *Allium cepa* L. at 5, 7.5, 10, and 20% concentration of leaf extract in five different time durations. It was found that higher concentration of leaf extract and longer duration of treatment generates the inhibitory effect on the division of cell. It also showed the change in phase indices and induced chromosomal abnormalities.

2.3 Herbicidal influences of Essential oil

Jimenezosornio *et al.* (1996) studied allelopathic potential of essential oil of *Chenopodium ambrosioides* L. on *Amaranthus hypochondriacus* L., and *Phaseolus acutifolius* A. Gray. It was found that *C. ambrosioides* essential oil inhibited germination of *A. hypochondriacus* by 50% at concentration of 0.552 µl /petri dish, while the hypocotyl growth of previously-germinated seeds of the same species was inhibited by 50% with 0.509 µL /petri dish. Ascaridole was the principal allelochemical; 0.098 µL /petri dish caused a 50% inhibition of *A. hypochondriacus* germination and 0.216 micro liter per petri dish inhibited hypocotyl growth of the same species by 50%. Another crop species, *P. acutifolius* A. Gray, grown hydroponically, was inhibited significantly with the lowest concentration of *C. ambrosioides* essential oil tested (0.15 ml/L). With higher concentrations, the primary root died and plant growth was visibly stunted.

Dudai *et al.* (1999) extracted essential oils from 32 aromatic plant species and evaluated for composition and allelopathic properties. They observed that volatile oils extracted from *Origanum syriacum* L., *Micromeria fruticosa* (L.) Druce, and *Cymbopogon citratus* (Nees) Stapf. inhibit germination of several weed species including wheat when applied at 20-80 ppm thus concluded could also be used as bioherbicides. Their effect however was depended on the type of soil.

Robles *et al.* (1999) made a study to assess allelopathic potentials of *Cistus albidus* L. essential oil for itself and *Lactuca sativa* L.. It was found that oils have little effect on

germination but significantly increased, even in low quantities number of nonviable seedlings and inhibit normal seedling growth.

Singh *et al.* (2002) conducted a study to assess the allelopathic effect of two volatile monoterpenes: cineole and citronellol on *Ageratum conyzoides* L. with a view to explore the possibility of their exploitation for future weed management. Both the monoterpenes severely affected the germination, speed of germination, seedling growth, chlorophyll content and respiratory activity. After two weeks of exposure, the weed plants wilted. Out of the two monoterpenes, cineole was more toxic in causing injury to the weed.

Tworkoski (2002) tested 25 plant-derived essential oils for herbicidal activity and found that those from red thyme (*Thymus vulgaris* L.), summer savory (*Satureja hortensis* L.), cinnamon (*Cinnamomum zeylanicum* Blume), and clove (*Syzygium aromaticum* [L.] Merr. et. Perry) were most toxic, causing cell death due to rapid electrolyte leakage on the detached leaves of dandelion (*Taraxacum officinale* Weber in Wiggers). Further, the application of 5 to 10% of these essential oils in combination with adjuvants caused the death of common lambsquarters, common ragweed, and johnsongrass within 1 day. Out of these four oils, the herbicidal activity of cinnamom oil was maximum, and this was attributed to the presence of eugenol that constituted 84% of the oil.

Salamci *et al.* (2007) tested the herbicidal effect of essential oils from *Tanacetum aucheranum* (Dc.) Schultz Bip. and *Tanacetum chiliophyllum* var. *chiliophyllum* (Fisch. Et Mey.) Schultz against seed germination and seedling growth of *Amaranthus retroflexus* L.*, Chenopodium album* L. and *Rumex crispus* L. and found that oils completely inhibited the germination and seedling growth relative to control.

2.4 Control of *Parthenium hysterophorus* using plant and plant's natural products as biocontrol agent

Joshi (1991) studied effect of *Cassia uniflora* Mill. on *Parthenium hysterophorus* in both laboratory level and field level. He found that phenolic leachates from different parts of *C. uniflora*, especially from germinating seeds significantly inhibited the germination of *P. hysterophorus* seeds and retarded the growth of seedlings from the successfully germinated *P.*

hysterophorus seeds. At field level, seedling of *C. uniflora* outcompeted the seedling of summer gerneration of *P. hysterophorus* causing reduction in height, dry weight and number of inflorescences of the latter which ultimately resulted in reduction of their seed output. Results indicated that *C. uniflora* replaces *P. hysterophorus* through interference.

Sinha and Singh (2004) studied allelopathic potential of *Xanthium strumarium* L. on *Parthenium hysterophorus* under laboratory condition. Fresh leaf extract of varied concentrations (0, 5, 10, 15, 20and 25%) was used to evaluate the allelopathic potency. Germination inhibition as well reduction in the root and shoot length and plant dry weight of the weed was highest with 25% *X. strumarium* leaf extract treatment.

Anjum *et al.* (2005) undertook a study to evaluate prospects of control of *Parthenium hysterophorus* by using crude preparation of *Imperata cylindrica* (L) Beauv.. It was found that aqueous extracts of both root and shoot of all applied concentrations of *I. cylindrica* significantly suppressed the germination and seedling growth. Inhibitory effect increased with increased concentration and shoot extract was more effective than root.

Singh *et al.* (2005) studied herbicidal potential of volatile oils from *Eucalyptus citriodora* Hook. against the noxious weed *Parthenium hysterophorus*. Laboratory bioassay done with varied concentration of oil from 0.2 to 5.0 nl ml^{-1} exhibited that seed germination and seedling length, chrorophyll content and respiratory activity of test plant decreased with increased concentration, germination being completely ceased at 5.0nl ml^{-1}. Further on post emergence bioassay it was found that visible damage increased and chlorophyll content and respiratory activity decreased with increased concentration in the 4 week seedlings pulverized with varied concentration of oil. It was found that eucalypt oil effect also on membrane integrity.

Javaid *et al.* (2006) evaluated herbicidal effect of aqueous root and shoot extract of three crops sunflower, sorghum and rice against germination and growth of *Parthenium hysterophorus*. Petridish bioassay with 5, 10, 15, 20, 25% (w/v) aqueous extracts of fresh plant materials of test crops indicated significant reduction effect on germination and root length. While foliar bioasssay with 50% and 100% (w/v) aqueous extract of sorghum and sunflower applied to 10 day *Parthenium* plant showed root biomass was significantly reduced by both concentration of both plant while shoot biomass though suppressed by both concentration of extracts was more effective for sunflower at lower concentration.

Pathipati *et al.* (2006) used aqueous leaf extract of the herb, *Breynia retusa* (Dennst.) Alston to study its herbicidal activity against four common weeds: *Calotropis gigantean* R. Br., *Parthenium hysterophorus* L. *Dathura meta*l L. and *Tridax procumbens* L. Foliar application of the water diluted extract caused chlorosis and necrosis in the treated plants. Crude leaf extract acted as contact herbicide. Changes in the plant growth, morphological aberrations, and color loss were the major changes. Extract initially killed the parts of the plant that were sprayed/exposed to extract but later the entire plant was defoliated. The extract was selective in herbicidal activity and did not affect rice (*Oryza sativa* L.) and wheat (*Triticum vulgare* ViII.) crops seed germination. The effects of extracts were also studied on RNA, DNA, proteins, free amino acids, glutamine, chlorophyll and ammonia. Thus *B. retusa* has allelopathic effects on several common weeds and may be developed as an effective weed control agent.

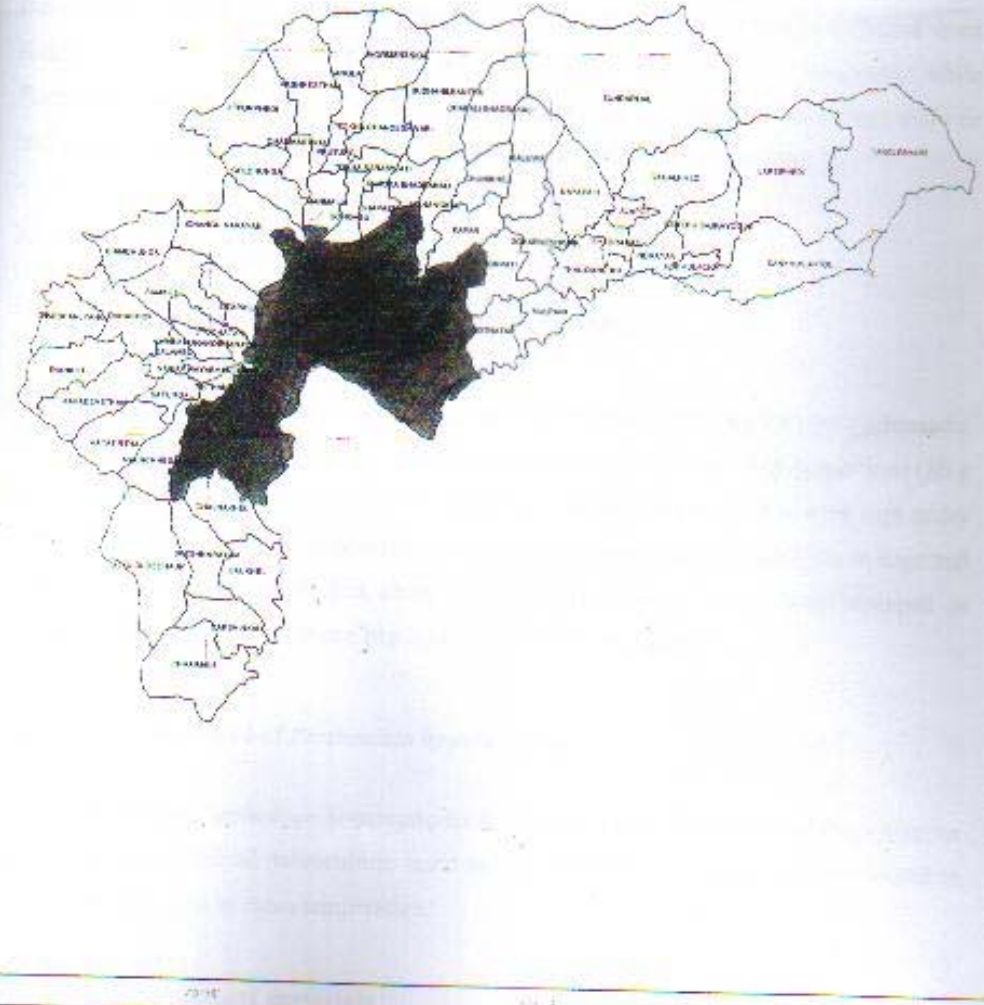
Riti and Singh (2006) studied the effects of leaf residue of *Croton bonplandianum* Baill. on growth and metabolism of *Parthenium hysterophorus*. The leaves size and seedling height of test plant was inhibited by leaf residues. The number of leaves, branches, capitula and seeds/plants were decreased. The inhibition of growth was due to decrease in chlorophyll, sugars, protein and lipid contents, while organic and amino acids were increased. Thus leaf residues of *C. bonplandianum* may be used to control the *P. hysterophorus*.

Poudel and Gupta (2007) studied herbicidal potential of essential oil of eucalyptus, lemon grass and camphor against germination and seedling vigour of *Parthenium hysterophorus*. They found that essential oil of all tested plant species was active to inhibit the germination and ceased it at higher concentration. However lemon grass oils showed herbicidal potential even at low concentration i.e. at 8×10^3 ppm.

KATHMANDU DISTRICT

ZONE : BAGMATI

District Code : 27



SCALE 1 : 250000

5000 0 5000 10000 Meters

LEGEND

- District Boundary
- VDC Bouncary
- MORANG District Name
- VDC Name

DISTRICT : KATHMANDU

Area : 395 Sq.Km (Approx.)

HORIZONTAL DATUM

Spheroid : Everest 1830
Projection : MUTM
Origin : Longitude 84° E , Latitude 0° N
False coordinates of origin : 500 000 m. Easting, 0 m. Northing
Scale Factor at Central Meridian : 0.9959

Map compiled from National Topographic Database at scales 1:25 000 and 1:50 000. Internal administrative boundaries are not demarcated on the ground. Map produced by the Survey Department, National Geographic Information Infrastructure Programme, (NGIIP) Kathmandu, 2003

LOCATION MAP

3. MATERIALS AND METHODS

3.1 Collection site

Collection site is situated in the Kathmandu valley (Latitude/Longitude-27°41'N / 85°19'E) which lies at 1300 m altitude and falls on sub-tropical climatic zone. Collection was made from Kalanki and Kirtipur area in the month of July 2007. *Artemisia* was collected from Kalanki area from fallow land (unused for years) where it was in natural occurrence while *Parthenium* weed was collected in Kirtipur area from road side where it maintained more or less monoculture stand naturally. The two sites are approximately 200 m apart.

3.2 Collection of plant material

3.2.1 Collection, drying and grinding of *Artemisia dubia* parts

Plant parts (stem, root and leaves) were collected randomly from Kalanki in Kathmandu. Those were immediately cleaned and chopped into small pieces. Few fresh proportions (50 g of each part) were taken to laboratory for experiment while other proportions were kept to dry in shade. Dried materials were grounded in mill separately and packed in polythene bags and stored for further utilization, and about 1.5 kg of shade dried leaves were wrapped in newspaper and stored in polythene bag for extraction of essential oil.

3.2 .2 Collection of seed of *Parthenium hysterophorus*

The noxious weed *Parthenium hysterophorus* seeds (test seeds) were collected from Kirtipur area where it maintained monoculture stand in July 2007, hand threshed and then stored in airtight bottle in dark at room temperature.

3.3 Extraction of plant materials

3.3.1 Extraction of plant grinded materials

Percolation method was followed for extraction of plant materials in different solvent in ecology laboratory of Central Department of Botany. For this 50 gm of plant (*Artemisia*

dubia) powder (leaf, stem and root) was kept in 500ml conical flask with 300ml solvent (Hexane, Methanol, Water and Chloroform) separately and left for 15 days. Thereafter the extract was filtered. To concentrate, filtrate was taken to the laboratory of Central Department of Chemistry and transferred in to the clean pre-weighed round bottom flask able to fit with rotatory vacuum evaporator. That round bottom flask containing filtrate to be concentrated was fitted with rotatory evaporator under negative pressure. The flask was constantly heated in rotating condition by using water bath below 80°C. Solvent was removed totally and the recovered solvent was collected in separate conical flask. Round bottom flask containing concentrated extract was weighed and result was noted. To find out weight of extract yield, flask without extract was subtracted from the weight of flask with extract. Same concentration process was repeated for all parts (leaf, stem and root) extracts extracted in different solvent viz. hexane, chloroform, methanol and water. These different parts were collected in sterilized well- capped bottle and placed in cool and dark place for further experimentation.

3.3.2 Extraction of essential oil

Extraction of essential oils from plant was done by hydro distillation method using Clevenger apparatus. For this, entire apparatus was washed with acetone and water and 50g of fresh shade dried plant leaf of *Artemisia dubia* was transferred to 1000 ml round bottom flask. It was hydro distillated with 500 ml of distilled water on a heating mantle with constant heating after setting the trap and water condenser. Distillation was continued until two consecutive reading taken at one hours intervals showed no change in oil content. Then the heat was removed and volume of oil was noted after 15 minutes. The water was drained off and oil was collected in a small capped bottle. It was diluted with 80% acetone and stock solution of essential oil of 20 % concentration was prepared by mixing 1ml of crude oil with 4 ml of acetone. It was stored in 4°C for further use.

3.4 Soil amendment with plant parts

Soil was collected from and *Parthenium hysterophorus* free area and then dried. Then sand soil mixture (1 volume sand + 2 volume soil) was prepared. To each 300 g of this soil 6, 12, 18, 24 and 30 g of powdered plant parts (leaf, stem and root) of *Artemisia dubia* was mixed respectively and separately to get the concentration of 2, 4, 6, 8 and 10 % (w/w). Such

amended soil was then stocked in polythene bags for further germination experiment of *P. hysterophorus* seeds.

3.5 Laboratory test of Allelopathy (Germination and early seedling growth bioassays)

3.5.1 Bioassay with extract

3.5.1.1 Bioassay with aqueous decomposed extract

10 g of shade dried and grinded material of different parts of *Artemisia dubia* was immersed in 100 ml of distilled water and kept for 24 h at room temperature. The extract was filtered and taken as 10% of stock aqueous dry part extract. Filtrate was diluted to varied concentration levels (10%, 8%, 6%, 4% and 2%) using distilled water. Test seeds were imbibed in distilled water for 36 h.

The petri dishes were sterilized with ethyl alcohol. The test seeds of *Parthenium hysterophorus* for germination were placed in petri dishes lined with single layer of filter paper supported by sterile absorbent cotton and moistened by 5 ml of different concentration of prepared test solution. Control set received distilled water. Each treatment was replicated thrice having 30 seeds in each petri dish. Petri dishes were kept in laboratory condition in room temperature and finally seed germination and root and shoot elongation were measured in twelfth day of sowing.

3.5.1.2 Bioassay with aqueous leachate extract

Leaching is one of important mechanism of releasing alleochemicals in the environment. It is escaping of chemical substances from plant by the action of rain, fog, snow and mist.

10 g of fresh plant materials (leaf, stem and roots) was collected randomly in vegetative stage in a sunny mid day of July 2007 and immersed in 100 ml of distilled water in beaker separately and kept for 24 h at room temperature and filtered using muslin cloth. This filtrate was taken as stock solution with 10% of concentration and different concentration i.e. 2%, 4%, 6%, 8% and 10% solution were made by dilution with distilled water and used for petri dish bioassay against test seeds with the same procedure stated above.

3.5.2 Bioassay with solvent extract

10 mg of different solvent extracts (hexane, methanol, chloroform and water) of different parts (leaf, stem and root) of the plant was re-dissolved in 1 ml of dimethyl sulphoxide (DMSO) in sterile vials and it was completely dissolved by continuous shaking. Then this solution was transferred to reagent bottle containing 99 ml of distilled water to make concentration of 1000 ppm and it was used as stock solution. Different concentrations (1000 ppm, 100 ppm, 10 ppm and 1 ppm) of solvent extracts were made by diluting stock solution with distilled water. Then viable seeds (imbibed for 36 h) of *P. hysterophorus* were allowed to germinate in sterilized petri dishes lined with filter paper supported with absorbent cotton and moistened in the different concentration of the extract. For control, 1% of DMSO solution was used.

Each treatment was replicated thrice with 30 seeds in each petridishes. After twelfth days, the germination percentage, and linear growth of root and shoot of seedling were measured.

3.5.3 Bioassay with essential oil

From the stock solution of 20 % concentration of essential oil of *Artemisia dubia*, varied concentration (20, 16, 12, 8 and 4 ml/L) were prepared by diluting with 80% acetone. 30 test seeds (imbibed for 36 h) of *Parthenium hysterophorus* were equidistantly placed in pre-soaked filter paper with 5 ml of water and supported by absorbent cotton lining sterilized petri dishes. To it 1 ml of prepared concentration of test solution of essential oil was added. The set was sealed and then kept in room temperature to facilitate germination. Same procedure was applied to triplicate number for each of prepared concentration of essential oil. Finally on the twelfth days, germination percentage and root, shoot length was measured.

3.5.4 Bioassay with plant parts amended soil

100g of soil amended with different plant parts powder were filled in disposable plastic plates. To it 20 test seeds (imbibed for 36 h) of *Parthenium hysterophorus* were sown. Each of triplicated treatment with varied concentration of amended soil was watered with 5 ml of

distilled water regularly and kept at room temperature. Finally germination and growth response was noted on the 28th day.

3.6 Statistical analysis

Significance of difference in root and shoot length of seedlings under different treatments were tested and compared separately using Duncan and Homogeneity test of one way Analysis of Variance (ANOVA). Statistical Package for Social Science (SPSS version 11.5, 2000) was used for analysis.

4. RESULTS

4.1 Yield of *Artemisia dubia*

4.1.1 Yield of crude extract of different parts of *Artemisia dubia* with different solvent

Shade dried powder of leaf; stem and root of *Artemisia dubia* was subjected to extraction with different solvent by percolation method for 15 days. Then the extract was filtered and filtrate was transferred to pre-weighted flask of rotatory vacuum evaporator for complete removal of solvent. After complete evaporation of solvent from the extract, weight of flask was taken and percentage yield was calculated which is given by figure 1.

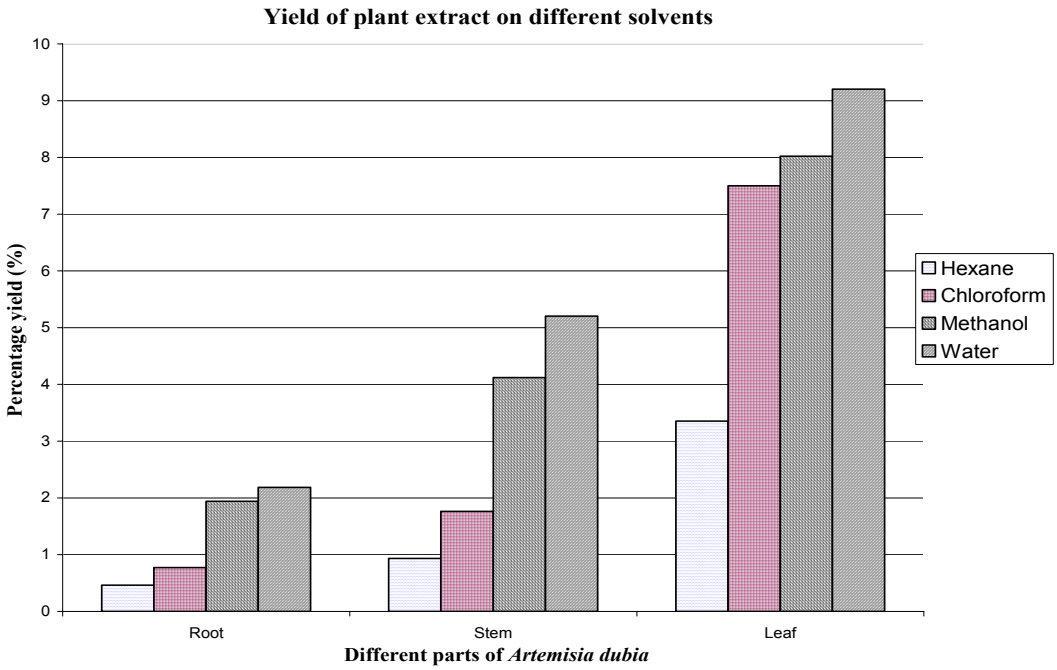


Figure 1: Yield Percentage of crude extract of different parts of *Artemisia dubia* with different solvent.

4.1.2 Yield of essential oil

Extraction of essential oils from foliar air dried part of *Artemisia dubia* using Clevenger apparatus produced 210 ± 12 µL volume of oil per 50 g making percentage yield of 0.42% (v/w).

4.2. Allelopathic effect of *Artemisia dubia* on *Parthenium hysterophorus*

4.2.1 Effect of extracts

4.2.1.1 Effect of aqueous decomposed extract

Germination of *Parthenium hysterophorus* was significantly affected by all the parts (leaf, stem and root) of *Artemisia dubia*. Inhibition increased with increasing concentration of extract leading to complete cessation of germination at highest concentration (10%) for stem. Leaf extract at all concentration imposed complete inhibition to the germination (Figure 2).

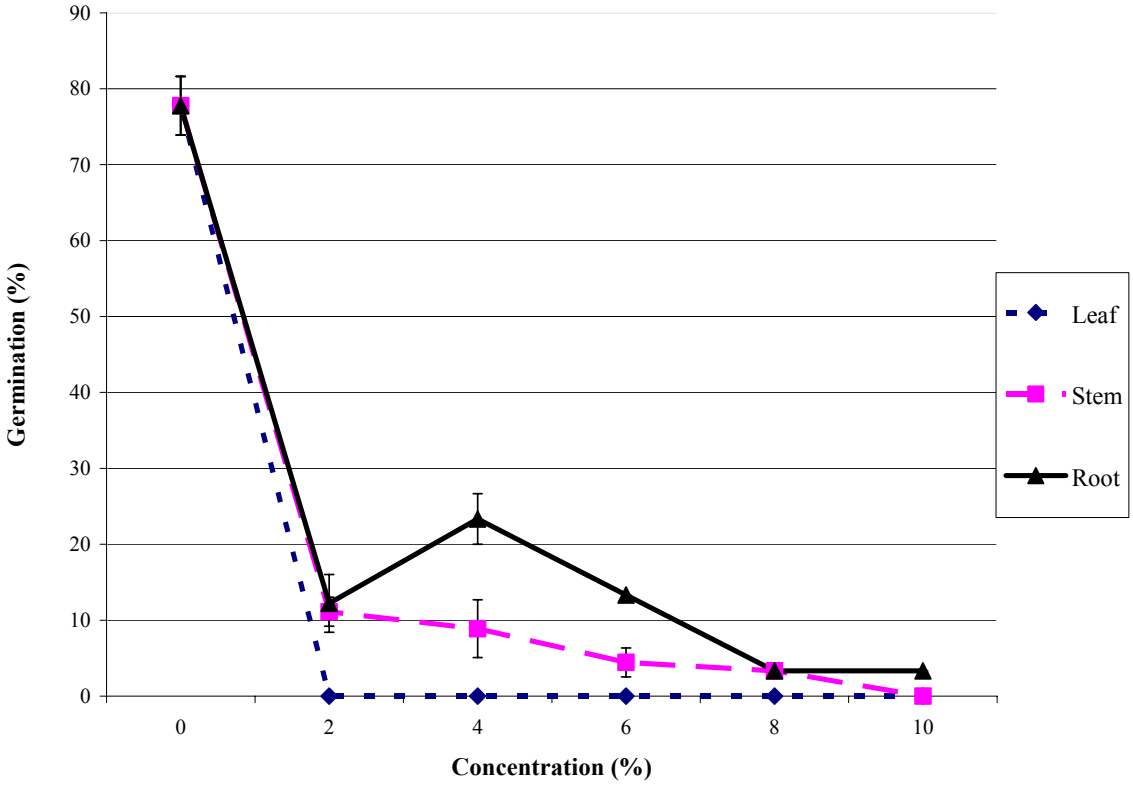


Figure: 2. Mean germination of *Parthenium hysterophorus* treated with various concentration of aqueous decomposed extract of leaf, stem and root of *Artemisia dubia* (Number of test seeds = 30; Bar indicate ± S.D.)

Extract of stem and root had varied effect on the seedling growth of ragweed. Stem extract on low concentration (2%) resulted in slight shoot elongation, however higher concentration led to decrease in shoot length. On the other hand root length was retarded by all the concentration of shoot extract. Root extract of *Artemisia dubia* resulted in retardation of both shoot and root length in all concentration compared to control with the effect being pronounced at higher concentration and on root length of seedling (Table 1).

Table: 1. Mean values of root and shoot length of *Parthenium hysterophorus* germinated in different concentration of aqueous decomposed extracts (Each value is the mean of samples)

Parts used	Concentration (%)	*Parthenium* seedling	
		Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
Leaf	2	-	-
	4	-	-
	6	-	-
	8	-	-
	10	-	-
Stem	2	1.55±0.2	2.5±0.23
	4	1.28±0.53	1.67±0.59
	6	1.22±0.20	1.15±0.12
	8	1.3±0.17	1.33±0.20
	10	-	-
Root	2	1±0.22	0.98±0.3
	4	1.22±0.28	1.11±0.41
	6	1.4±0.34	0.9±0.23
	8	0.8±0.28	0.85±0.35
	10	1±0.1	0.83±0.23
Control	0	1.53±0.31	3.65±0.63

Note: - = No germination

4.2.1.2 Effect of aqueous leachate extract

Allelopathic effect was different for different parts at different concentration. Germination inhibition showed a varied pattern. Stem extract even at lower concentration resulted in high inhibition. Like wise root extract showed greater inhibition at medium concentration (4%) but it's concentration of 6% resulted in abrupt increase in germination. However general pattern for all the parts extract was inhibition in highest concentration (Figure 3).

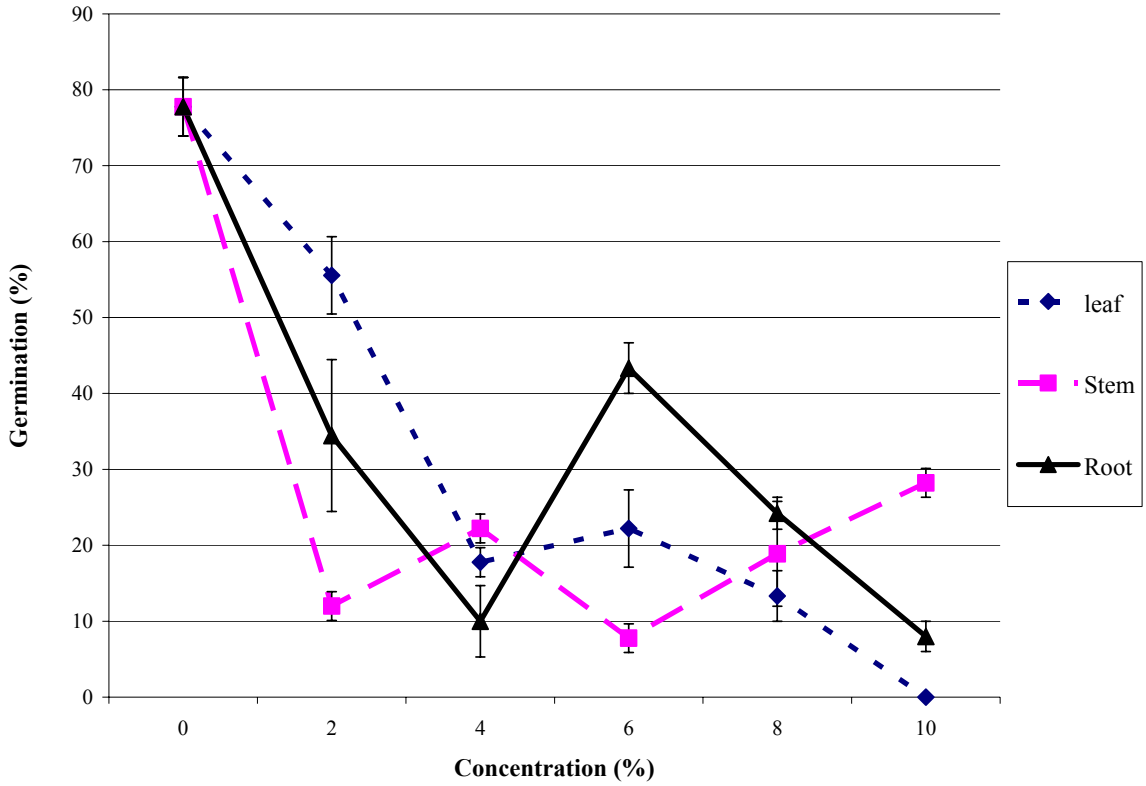


Figure: 3. Mean germination of *Parthenium hysterophorus* treated with various concentration of aqueous leachate extract of leaf, stem and root of *Artemisia dubia* (Number of test seeds = 30; Bar indicate ± S.D.)

There was significant difference in mean values of root and shoot length of ragweed germinated in different treatment of all the parts (Table 2).

Table: 2. Analysis of variance of effect of aqueous leachate extracts of different parts of *Artemisia dubia* on shoot and root length of *Parthenium hysterophorus* seedling

Effect of leaf extract						
Source of variance	Stem Length			Root length		
	d.f.	F	P	d.f.	F	P
Between groups	3	15.210	.000	3	129.225	.000
Within groups	152			152		
Total	155			155		
Effect of stem extract						
Between groups	4	28.89	.000	4	47.00	.000
Within groups	136			136		
Total	140			140		
Effect of root extract						
Between groups	3	14.38	.000	3	45.98	.000
Within groups	150			150		
Total	153			153		

Note: d.f. = degree of freedom, P = level of significance

Leaf leachate extract resulted in high retardation of root length compared to shoot length. Stem extract showed promotory effect on shoot length and it was maximum for higher concentrations, on the other hand root length was inhibited compared to control but it was independent of concentration. All concentrations of root leachate extract resulted in elongation of shoot length, but effect on root length was less significant (Table 3).

Table: 3. Mean values of root and shoot length of *Parthenium hysterophorus* germinated in different concentration of leachate extracts (Each value is the mean of samples; significance difference is shown by different letters at $\alpha = 0.05$)

Effect of leaf extract		
Treatment (Concentration in percentage)	*Parthenium* seedling	
	Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
Control	1.53±0.31 c	3.65±0.63 c
2%	1.33±0.25 b	3.07±0.46 b
4%	1.03±0.36 a	1.45±0.37 a
6%	1.24±0.27 b	1.56±0.33 a
8%	1.3±0.24 *	1.56±0.25 *
10%	-	-
Effect of stem extract		
Control	1.53±0.31 a	3.65±0.63 b
2%	1.37±0.19 a	2.25±0.51 a
4%	1.81±0.30 b	2.41±0.61 a
6%	1.52±0.44 *	2.06±0.43 *
8%	2.03±0.27 c	2.14±0.60 a
10%	2.19±0.34 c	2.13±0.61 a
Effect of root extract		
Control	1.53±0.31 a	3.65±0.63 b
2%	1.45±0.39 a	2.73±0.60 a
4%	1.95±0.28 *	2.48±0.46 *
6%	1.78±0.30 b	2.42±0.47 a
8%	2.06±0.52 c	2.55±0.51 a
10%	1.44±0.19 *	1.98±0.36 *

Note: - = No germination; * = Excluded from statistiscal analysis (small sample size)

4.2.2 Effect of solvent extracts

4.2.2.1 Effect of hexane extract

Germination was inhibited by hexane fraction of all parts extract at highest concentration (1000 ppm). At lower concentration, it was promoted by leaf and root extract. Stem extract exhibited slight inhibition initially, but subsequently at medium concentrations resulted in promotion of germination (Figure 4).

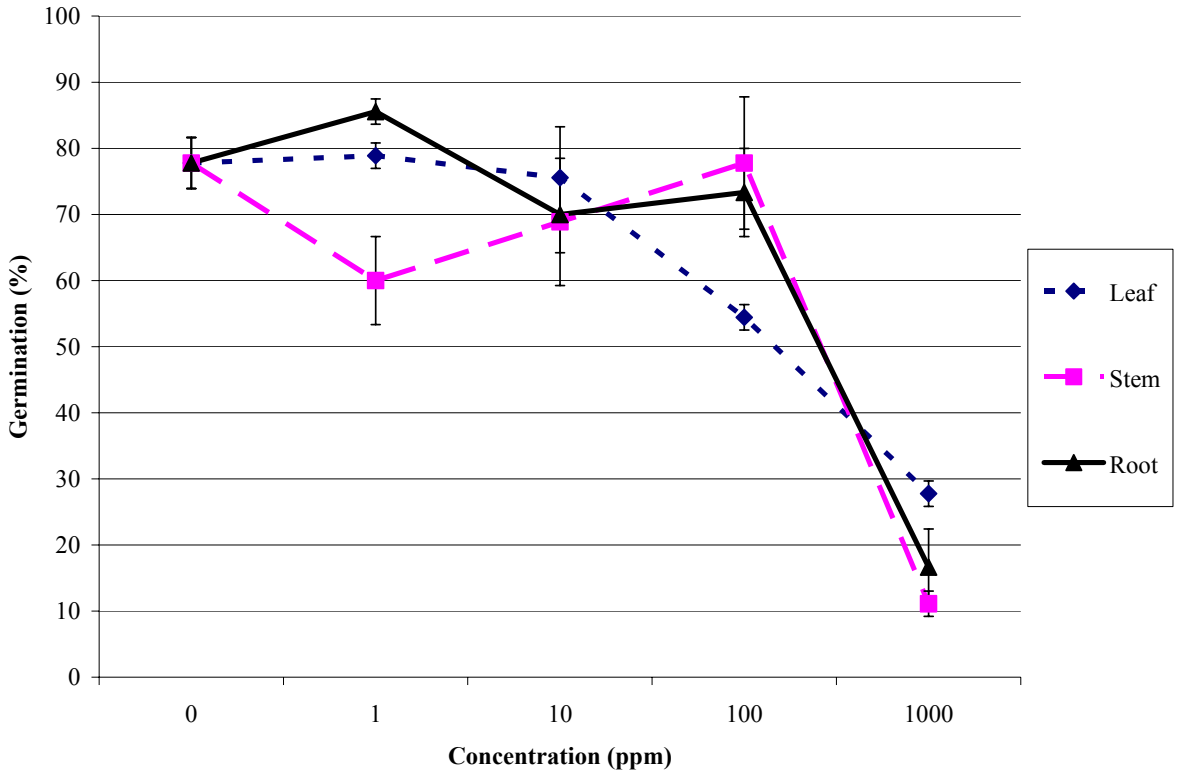


Figure: 4. Mean germination of *Parthenium hysterophorus* treated with various concentration of hexane extracts of leaf, stem and root of *Artemisia dubia* (Number of test seeds = 30; Bar indicate ± S.D.)

ANOVA performed on shoot and root length of *Parthenium hysterophorus* treated with different concentration of extracts of varied parts of *Artemisia dubia* showed that there was significant difference in mean values of root due to root extract however only stem extract treatment produced significant difference in shoot length (Table 4). The hexane extract of all parts significantly reduced root elongation with this being highly significant to leaf extract compared with stem and root. Leaf extract resulted in inhibitory effect in shoot elongation at higher concentration but stem extract had promotory effect. There were homogenous variances in root extract treatment in shoot length of ragweed compared to control (Table 5).

Table: 4. Analysis of variance of effect of hexane extracts of different parts of *Artemisia dubia* on shoot and root length of *Parthenium hysterophorus* seedling

Effect of leaf extract						
Source of variance	Stem length			Root length		
	d.f.	F	P	d.f.	F	P
Between groups	4	3.88	.004	4	62.06	.000
Within groups	267			267		
Total	271			271		
Effect of stem extract						
Between groups	4	14.17	.000	4	57.05	.000
Within groups	255			255		
Total	259			259		
Effect of root extract						
Between groups	4	1.80	.129	4	22.65	.000
Within groups	268			268		
Total	272			272		

Note: d.f. = Degree of Freedom, P = Level of Significance

Table: 5. Mean values of root and shoot length of *Parthenium hysterophorus* germinated in different concentration of hexane extracts (Each value is the mean of samples; significance difference is shown by different letters at $\alpha = 0.05$)

Effect of leaf extract		
Treatment (Concentration in ppm)	*Parthenium* seedling	
	Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
Control	1.14±0.1 a	3.61±0.6 d
1	1.10±0.20 a	3.48±0.81 d
10	1.19±0.20 ab	3.15±0.60 c
100	1.28±0.35 b	2.15±0.42 b
1000	1.16±0.23 a	1.85±0.38 a
Effect of stem extract		
Control	1.14±0.1 ab	3.61±0.6 d
1	1.09±0.19 a	3.44±0.71 d
10	1.26±0.32 bc	3.03±0.54 c
100	1.41±0.31 d	2.32±0.43 b
1000	1.34±0.25 cd	1.96±0.25 a
Effect of root extract		
Control	1.14±0.1 a	3.61±0.6 c
1	1.15±0.22 a	3.41±.06 c
10	1.20±0.20 a	3.12±0.5 b
100	1.23±0.22 a	2.65±0.6 a
1000	1.22±0.27 a	2.68±0.61 a

4.2.2.2 Effect of chloroform extract

Treatment with chloroform extract resulted in inhibition in germination. Inhibition increased with increased concentration for all the parts extract (Figure 5).

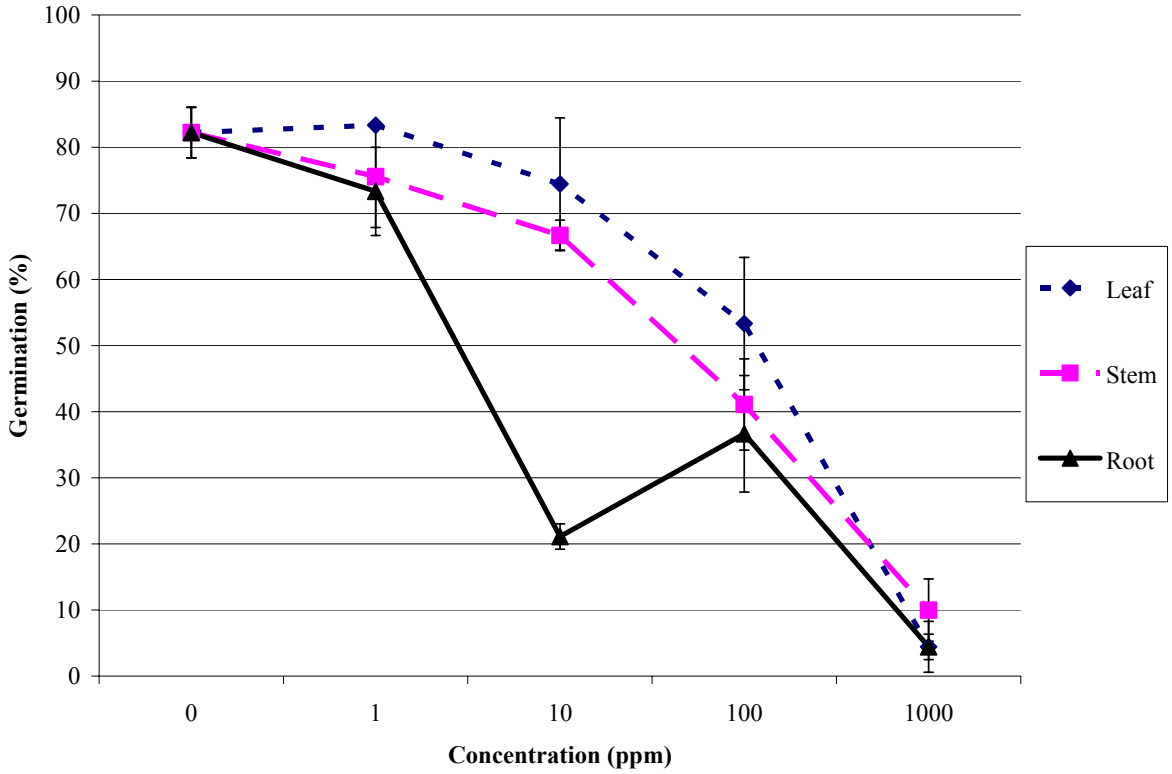


Figure: 5. Mean germination of *Parthenium hysterophorus* treated with various concentration of chloroform extract of leaf, stem and root of *Artemisia dubia* (Number of test seeds = 30; Bar indicate ± S.D.)

There was no significant difference in mean values of shoot length when treated with root and leaf extract; shoot extract treatment resulted in significant difference. On the other hand effect of all parts extracts produced significant effect on root length (Table 6).

Inhibitory effect on root length was significant with heterogeneity in variance for treatment with all parts extract but homogeneity in variance was observed in shoot length when treated with root and leaf extract. Stem extract on the other hand had significant promotory effect in shoot length (Table 7).

Table: 6. Analysis of variance of effect of chloroform extracts of different parts of *Artemisia dubia* on shoot and root length of *Parthenium hysterophorus* seedling

Effect of leaf extract						
Source of variance	Stem length			Root length		
	d.f.	F	P	d.f.	F	P
Between groups	3	2.53	.057	3	86.94	.000
Within groups	256			256		
Total	259			259		
Effect of stem extract						
Between groups	3	30.4	.000	3	33.72	.000
Within groups	229			229		
Total	232			232		
Effect of root extract						
Between groups	3	5.04	.002	3	32.09	.000
Within groups	222			222		
Total	225			225		

Note: d.f. = Degree of Freedom, P = Level of Significance

Table: 7. Mean values of root and shoot length of *Parthenium hysterophorus* weed germinated in different concentration of chloroform extracts (Each value is the mean of samples; significance difference is shown by different letters at $\alpha = 0.05$)

Effect of leaf extract		
Treatment (Concentration in ppm)	*Parthenium* seedling	
	Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
Control	1.10±0.21 a	3.69±0.64 d
1	1.16±0.24 ab	3.47±0.66 c
10	1.21±0.23 b	2.64±0.57 b
100	1.12±0.25 b	2.06±0.56 a
1000	0.53±0.05 *	1.2±0.1 *
Effect of stem extract		
Control	1.10±0.21 a	3.69±0.64 d
1	1.18±0.31 a	3.40±0.65 c
10	1.37±0.33 b	2.90±0.53 b
100	1.66±0.36 b	2.55±0.61 a
1000	1.5±0.2 *	2.16±0.26 *
Effect of root extract		
Control	1.10±0.21 a	3.69±0.64 c
1	1.13±0.26 a	3.56±0.64 c
10	1.3±0.27 b	2.99±0.62 b
100	1.16±0.3 a	2.53±0.67 a
1000	0.7±0.20 *	1.6±0.11 *

Note: * = Excluded from statistical analysis (small sample size)

4.2.2.3 Effect of methanol extract

Germination was inhibited by the methanol *fraction of Artemisia dubia* extract from all parts compared to control however the pattern was varied at other concentration level. After initial inhibition at concentration of 1 ppm, both root and stem extract resulted in slight promotion at medium concentration. Leaf extract resulted in significant inhibition of germination compared to stem and root extract (Figure 6).

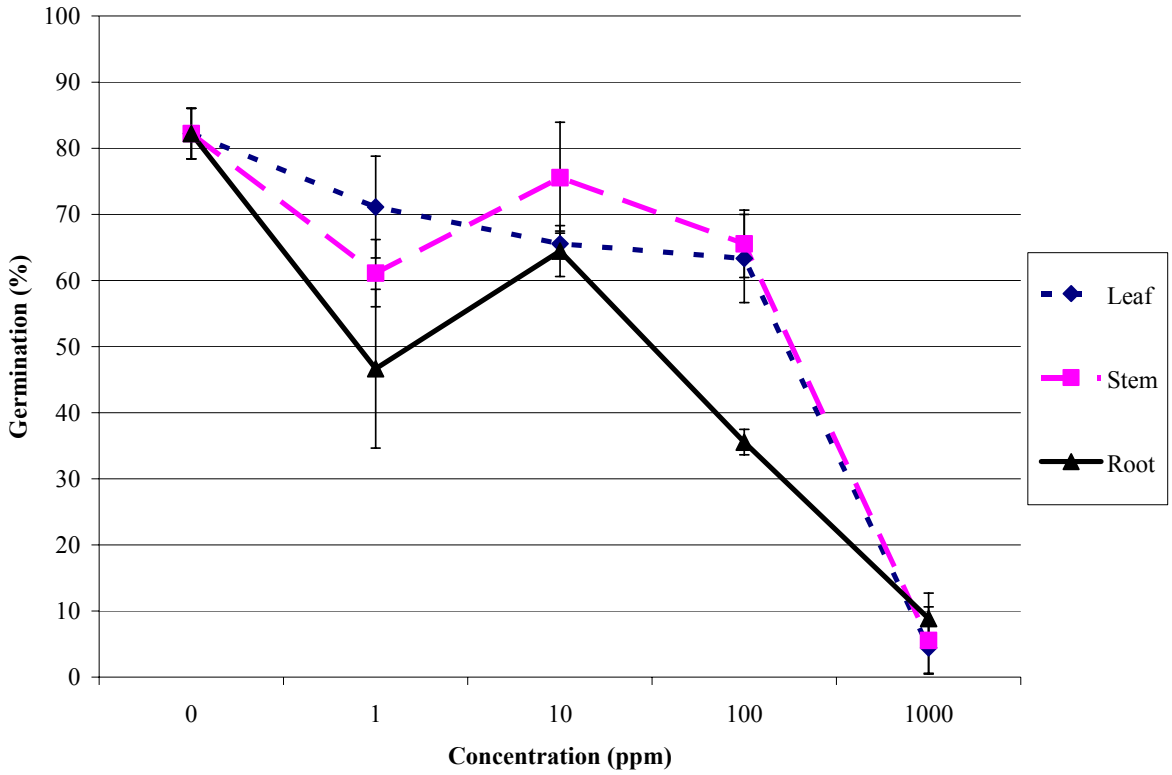


Figure: 6. Mean germination of *Parthenium hysterophorus* treated with various concentration of methanol extract of leaf, stem and root of *Artemisia dubia* (Number of test seeds = 30; Bar indicate ± S.D.)

ANOVA test showed that root length of seedling was significantly affected by treatment with all the parts however effect on shoot was not pronounced except in treatment with shoot extract (Table 8).

Table: 8. Analyses of variance of effect of methanol extracts of different parts of *Artemisia dubia* on shoot and root length of *Parthenium hysterophorus* seedling

Effect of leaf extract						
Source of variance	Stem length			Root length		
	d.f.	F	P	d.f.	F	P
Between groups	3	4.44	.005	3	64.34	.000
Within groups	246			246		
Total	249			249		
Effect of stem extract						
Between groups	3	7.44	.000	3	49.46	.000
Within groups	248			248		
Total	251			251		
Effect of root extract						
Between groups	3	1.41	.239	3	52.3	.000
Within groups	198			198		
Total	201			201		

Note: d.f. = Degree of Freedom, P = Level of Significance

Variance in effect was seen in all parts extract when compared among the different concentrations of treatment on root length which was inhibition to root elongation. Homogenity in variance was observed in shoot length in treatment with leaf and root extract but shoot extract showed significant elongation compared to control (Table 9).

Table: 9. Mean values of root and shoot length of *Parthenium hysterophorus* germinated in different concentration of methanol extracts (Each value is the mean of samples; significance difference is shown by different letters at $\alpha = 0.05$)

Effect of leaf extract		
Treatment (Concentration in ppm)	*Parthenium* seedling	
	Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
Control	1.10±0.21 a	3.69±0.64 c
1	1.12±0.21 ab	3.10±0.64 b
10	1.20±0.23 b	2.50±0.68 a
100	1.05±0.27 a	2.32±0.48 a
1000	0.65±0.23 *	1.3±0.14 *
Effect of stem extract		
Control	1.10±0.21 a	3.69±0.64 c
1	1.23±0.32 b	3.11±0.69 b
10	1.31±0.30 b	2.62±0.66 a
100	1.31±0.34 b	2.44±0.57 a
1000	1.1±0.39 *	1.66±0.54 *
Effect of root extract		
Control	1.10±0.21 a	3.69±0.64 c
1	1.10±0.28 a	3.13±0.57 b
10	1.09±0.28 a	2.56±0.59 a
100	0.99±0.29 a	2.38±0.55 a
1000	0.7±0.23 *	1.48±0.29 *

Note: * = Excluded from statistiscal analysis (small sample size)

4.2.2.4 Effect of aqueous extract

Germination was highly inhibited by all parts of *Artemisia dubia* water extract. The effect increased with increasing concentration and leaf extract resulted in maximum inhibition compared to stem and root (Figure 7).

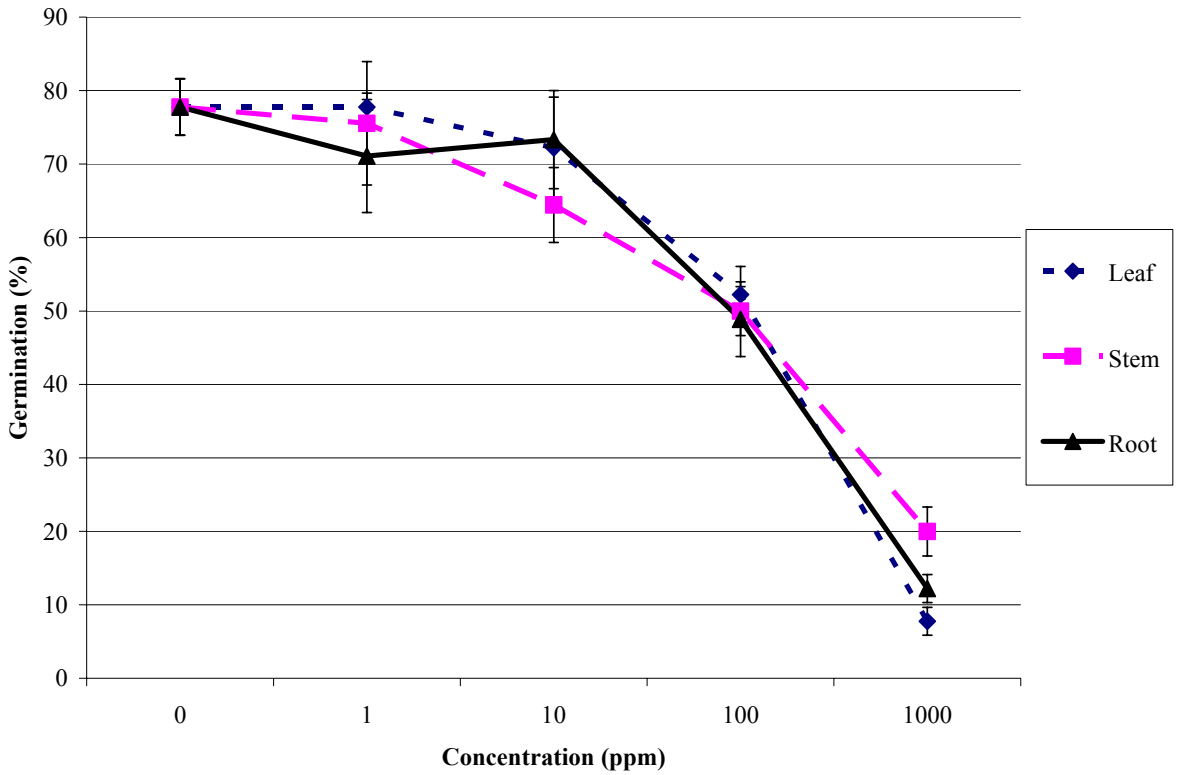


Figure: 7. Mean germination of *Parthenium hysterophorus* treated with various concentration of aqueous extract of leaf, stem and root of *Artemisia dubia* (Number of test seeds = 30; Bar indicate ± S.D.)

Aqueous fraction of all parts showed strong inhibitory effect on root length elongation with heterogeneity in variance when compared among treatments with different concentrations. Stem extract resulted in slight elongation in shoot length at lower concentration (10 ppm); root fraction treatment showed no difference in shoot length compared to control in all concentration except higher concentration which led to retardation in shoot length. Significant retardation in shoot length was observed for both stem and leaf extract at higher concentration though it was not pronounced at lower concentration (Table 11).

Table: 10. Analysis of variance of effect of aqueous extract of different parts of *Artemisia dubia* on shoot and root length of *Parthenium hysterophorus* seedling

Effect of leaf extract						
Source of variance	Stem length			Root length		
	d.f.	F	P	d.f.	F	P
Between groups	3	2.45	.064	3	34.62	.000
Within groups	227			227		
Total	230			230		
Effect of stem extract						
Between groups	4	4.25	.002	4	42.73	.000
Within groups	249			249		
Total	253			253		
Effect of root extract						
Between groups	4	7.79	.000	4	46.14	.000
Within groups	248			248		
Total	252			252		

Note: d.f. = Degree of Freedom, P = Level of Significance

Table: 11. Mean values of root and shoot length of *Parthenium hysterophorus* germinated in different concentration of aqueous extracts (Each value is the mean of samples; significance difference is shown by different letters at $\alpha = 0.05$)

Effect of leaf extract		
Treatment (Concentration in ppm)	*Parthenium* seedling	
	Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
Control	1.14±0.20 ab	3.61±0.68 d
1	1.09±0.21 a	3.29±0.57 c
10	1.19±0.27 b	2.74±0.71 b
100	1.08±0.26 a	2.49±0.48 a
1000	0.67±0.22 *	1.27±0.34 *
Effect of stem extract		
Control	1.14±0.20 ab	3.61±0.68 d
1	1.13±0.20 ab	3.55±0.63 d
10	1.27±0.37 c	3.17±0.72 c
100	1.18±0.29 c	2.65±0.92 b
1000	1.02±0.17 a	1.46±0.31 a
Effect of root extract		
Control	1.14±0.20 b	3.61±0.68 d
1	1.16±0.21 b	3.48±0.57 d
10	1.12±0.20 b	3.18±0.63 c
100	1.11±0.24 b	2.51±0.57 b
1000	0.77±0.31 a	1.45±0.45 a

Note: * = Excluded from statistical analysis (small sample size)

4.2.3 Effect of essential oil

Essential oil of *Artemisia dubia* was found to be highly inhibitory to the germination. It was pronounced even at lower concentration (4ml/L). Inhibition increased with increasing concentration (Figure 8).

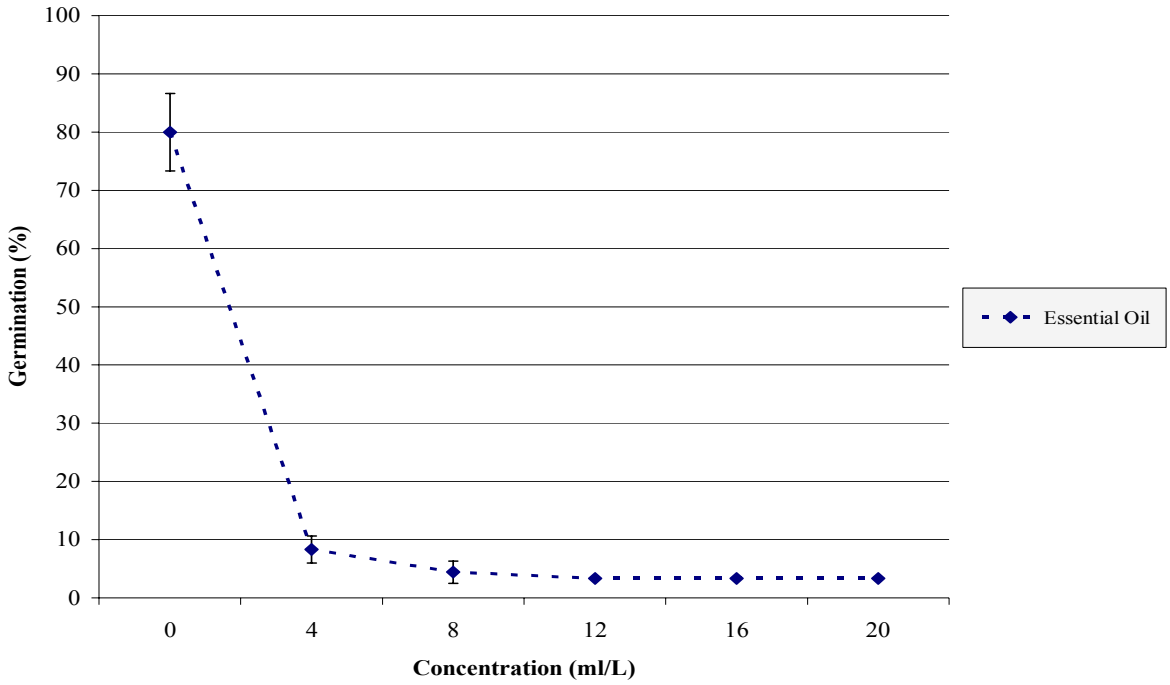


Figure: 8. Mean germination of *Parthenium hysterophorus* treated with various concentration of essential oil of *Artemisia dubia* (Number of test seeds = 30; Bar indicate ± S.D.)

There was significant reduction in shoot and root length of seedling when compared to that of control. Reduction was proportional to the concentration of applied concentrations of oil (Table 12).

Table: 12. Mean values of root and shoot length of *Parthenium hysterophorus* germinated in different concentration of essential oil (Each value is mean of samples)

Effect of Essential Oils		
Treatment concentration (ml/L)	*Parthenium* Seedling	
	Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
4	0.88±0.27	0.64±0.19
8	0.7±0.21	0.87±0.26
12	0.6±0	0.65±0.07
16	0.45±0.07	0.35±0.07
20	0.3±0	0.2±0
Control	1.21±0.25	1.93±0.30

4.2.4 Effect of plant parts amended soil

Plants parts amended soil had inhibitory effect on germination of ragweed seed with leaf being highly inhibitory compared to shoot and root. Germination followed complete cessation beyond 4% concentration of soil amended with leaf parts (Figure 9).

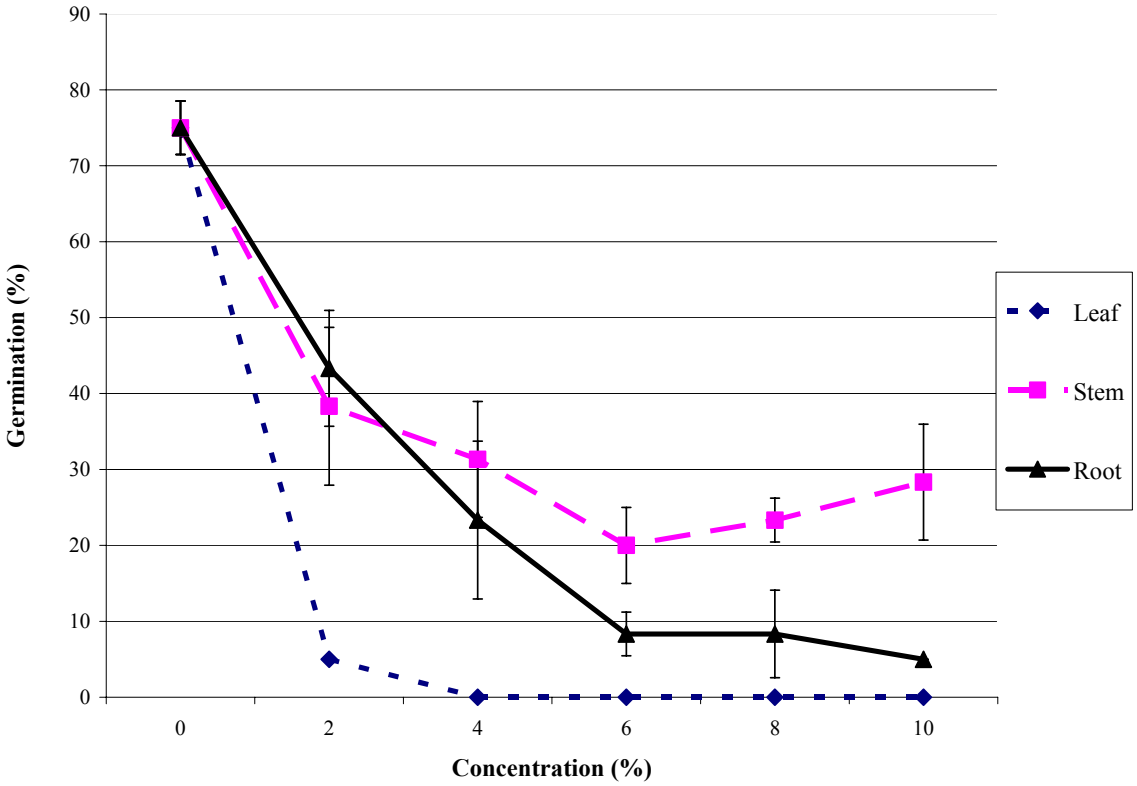


Figure: 9. Mean germination of *Parthenium hysterophorus* treated with various concentration of soil amended with leaf, stem and root of *Artemisia dubia* (Number of test seeds = 20; Bar indicate ± S.D.)

Root length and shoot length elongation was also highly affected compared to control however stem elongation was more inhibited by root parts amended soil compared to shoot parts amended soil (Table 13).

Table: 13. Mean values of root and shoot length of *Parthenium hysterophorus* germinated in different concentration of soil amended with different parts of *Artemisia dubia*

Parts used	Concentration (%)	*Parthenium* seedling	
		Shoot Length(cm)± S.D.	Root Length(cm)± S.D.
Leaf	2	1.33±0.35	0.66±0.15
	4	-	-
	6	-	-
	8	-	-
	10	-	-
Stem	2	1.75±0.31	2.62±0.52
	4	1.86±0.39	1.90±0.49
	6	2±0.52	1.81±0.39
	8	2.05±0.31	1.83±0.40
	10	2.22±0.48	1.93±0.41
Root	2	1.67±0.32	3.17±0.66
	4	1.58±0.33	2.37±0.45
	6	1.41±0.24	2.3±0.48
	8	1.73±0.46	2.56±0.20
	10	1.1±0.14	1.15±0.49
Control	0	3.08±0.58	3.68±0.70

Note: - = No germination

5. DISCUSSION

5.1 Yield of *Artemisia dubia*

The yield of dried powder extract was highest in highly polar solvent (water) and was lowest in non-polar solvent (hexane). Extract was highest for the leaf part compared to stem and root. This is due to drawing of soluble pigments in extract. The fact besides the high amount of extraction with water solvent may be due to presence of water-soluble polar compounds present in plant according to the principle "like dissolves like". This result comes to coherence to the earlier findings reported by Poudel (2004) for *Artemisia dubia* and Suwal (2006) for *Chromoleana odorata* (L.) King and Robinson.

Essential oil extraction followed by hydro distillation resulted in yield of 210 ± 12 µL volume of oil per 50 g of shade dried leaf.

5.2 Allelopathic effect of *Artemisia dubia* on *Parthenium hysterophorus*

5.2.1 Effect of different extract of *Artemisia dubia* on the germination of test seeds of *Parthenium hysterophorus*

There was significant inhibitory effect on germination of *Parthenium* seed by different solvent extract, aqueous decomposed extract and leachate and it was highly pronounced in higher concentration, while the extract at lower concentration at some instances even promoted germination. This may be because only higher concentration was able to cause the death of the embryo. Similar finding was reported by Escudero *et al.* (2000) when they tested seed germination of *Helianthemum squamatum* and *Lactuca sativa* against varied concentration of aqueous extract of *Artemisia herba-alba*. Inhibition was higher for leaf extract compared to stem and root extract and this may be because leaf contains the repository of inhibitory compounds. This result is comparable to earlier findings that reported more harmful effect of leaf extract than shoot and root extract (Poudel, 2004; Suwal, 2006).

Essential oil also reduced the germination noticeably when compared to control with effect being more pronounced at higher concentration. This comes as support to reporting of Muller

et al. (1964) who found that volatile foliage compounds were active ingredients causing repression in growth of other plants in vicinity of *Artemisia californica* Less.. Similar finding was reported for the identified or non identified compounds in essential oil/volatile materials of different *Artemisia* species (Dudai *et al.*, 1999; Barney *et al.*, 2005; Karban, 2007).

Soil amended with plant parts also led to inhibition of germination with leaf imparting more effect. And inhibition was dose dependent. Delabays (2000) had reported similar finding with reduction in weed emergence on field trials while incorporating dry leaves of artemisinin rich *Artemisia annua* in soil.

5.2.2 Effect on seedling growth of test seed of *Parthenium hysterophorus*

The allelopathic influences of different parts extract of *Artemisia. dubia* on *Parthenium hysterophorus* seedling vigour (shoot/root length) was varied and it was dependent on concentration and type of solvents (hexane, chloroform, methanol, water) used for extraction. All parts extract (solvent/leachate/decomposed) resulted in the retardation of root length but inhibition of shoot length elongation was observed only on few instances. This strong effect on root in comparison to shoots may have caused because of roots direct contact with extract and consequently this is comparable to earlier findings reported by Bhowmik and Doll (1984) and Rice (1984).

All the tested four organic solvent (hexane, chloroform, methanol and water) extract had attributed the significant and similar effects on germination and seedling vigour of *Parthenium hysterophorus*. This is against the earlier findings of Malla (2003) on *Ageratum conyzoids*. This could be because allelopathic interference of allelochemicals is selective in nature.

Aqueous decomposed extract of leaf even in low concentration of 2% lead to complete cessation of germination and effect was also inhibitory for stem and root decomposed extract. While in aqueous leachate extract both root and shoot leachate led to elongation of ragweed seedling stem. This could be because, in decomposed extract allelopathic compounds leached out completely from the dust of parts prepared while in aqueous leachate extract, live tissue (without maceration) were immersed in water so chemicals that got leached from the

unexposed cells were in the concentration level that was promotory. Lovett (1989) has reported that biological activities of receiver plants to allelochemicals are known to be concentration dependent with a response threshold. Responses are, characteristically, stimulation at low concentrations of allelochemicals, and inhibition as the concentration increases.

Effect had followed the order of leaf > stem > root, where all solvent extract of all parts resulted in root retardation but stem extract except in aqueous fraction has resulted in promotory effect on shoot length elongation. Similar concentration dependent and differential effect was reported by Mo *et al.* (2005) due to aqueous extract of *Lactarius hatsudake* N. Tanaka on rice which was stimulation on shoot growth but inhibition on root growth.

Soil amended with leaf part powder exerted high inhibition to the emergence of *Parthenium hysterophorus* seedling and delayed the germination, this could be because the chemical effect of phytotoxins released from decomposition of residues also contribute to selective weed control (Borgos and Talbert, 2000; Nagabhushana *et al.* 2001) and allelochemicals are strongly influential in earlier days of their release by decomposition but slowly after degradation their inhibition is markedly reduced. This effect is comparable to the findings of Xuan *et al.* (2005), which reported drastic reduction in magnitude of inhibition induced by incorporating allelopathic plants powder to soil after 20-25 days compared to earlier days.

Essential oil was observed to impose a significant effect on germination and seedling vigor of *P. hysterophorus.* Several monoterpenes and sesquiterpene lactones are reported to be present in volatile compounds of *Artemisia* spp. (Khalilov *et al*., 2001; Kordali *et al.*, 2006) and are known to be potential allelochemicals. Kohli *et al.* (1998) had demonstrated that essential oils of Eucalyptus spp. rich in volatile monoterpenes have a potential to manage ragweed *(Parthenium*). On the other hand, several sesquiterpene lactones are known to be phytotoxic and these belong to terpenoid group of allelochemicals (Singh *et al.*, 2003).

Artemisinin: a sesquiterpene lactone which is water insoluble is reported to be promising phytotoxic substance reported from *Artemisia annua* which is one member to the genus *Artemisia* (Duke *et al.*, 1987; Chen and Leather, 1990).

Poudel (2004) has reported presence of saponins and tanins in aqueous fraction of *Artemisia dubia*. Saponins are natural group of compounds known for its wide range of allelopathic activity (Olezek *et al.* 1992; Waller *et al.*, 1992, 1995) and as a promising source that could be used as herbicides (Sondhia and Saxena, 2003). Tanins are complex molecules, which reduce the digestibility of plant tissue by herbivores; however there are several hydrolysable tannins that are known to be growth and germination inhibitor (Harborne, 1984). Lydon *et al.* (1997) has suggested that other molecules may also be involved for the phytotoxic effect as they found aqueous extract devoid of artemisinin to be equally phytotoxic as compared to treatment with artemisin.

Besides, flavonoids and phenolics (known group of allelochemicals) are also reported from some of *Artemisia* spp (Suleimenov *et al.*, 2005; Modallal and Al-Charchafchi, 2006).

These all suggest *Artemisia dubia* to be repository of both polar and non polar allelochemicals that could be exploited for the control of ragweed and further provide conformity to its herbicidal potentiality.

6. CONCLUSION

Based on result, the major conclusions drawn are listed below:

- The crude extract of *Artemisia dubia* has been found according to the type of part used and type of solvent used in extraction. The yield obtained was in the following trend
  Leaf>Stem>Root (Part wise)
  Aqueous> Methanol> Chloroform> Hexane
  The yield of essential oils extracted by steam distillation was found to be 0.42% (v/w).

- All of the solvent extract (water, methanol, chloroform, and hexane) of *Artemisia dubia* resulted in similar effect on germination and seedling growth of *Parthenium hysterophorus*.

- Omitting some exceptions in lower concentrations, all treatments (aqueous decomposed/leachate extract, solvent extract), essential oil and plant parts amended soil) was found inhibitory being more pronounced in accordance with the increasing concentration.

- Among all the extracts leaf extract has been found to impose more inhibitory effect.

- Root length of seedling was retarded in almost all cases while stem length experienced no effect; some times elongation (stem and root extract of leachate, stem extract of hexane, chloroform); and some times retardation (aqueous decomposed leaf extract, essential oil).

- Aqueous decomposed extract of leaf, leaf amended soil and essential oil fraction was found to be most effective for ragweed.

7. RECOMMENDATION

From the study undertaken and observed result following recommendations are made:

- This research work is laboratory based preliminary screening of allelopathic influences of *Artemisia dubia* against *Parthenium hysterophorus*. This should be replicated in field conditions.

- *Artemisia* has repository of potential chemical to be used for weed control, this attribute may be involved in developing natural herbicides. Further research should be carried considering this fact.

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Plate 1

Plate 1 A: Different parts of *Artemisia dubia.*

Plate 1 B: Different parts powder of *A. dubia*

Plate 1C: Extraction in different solvents by percolation method

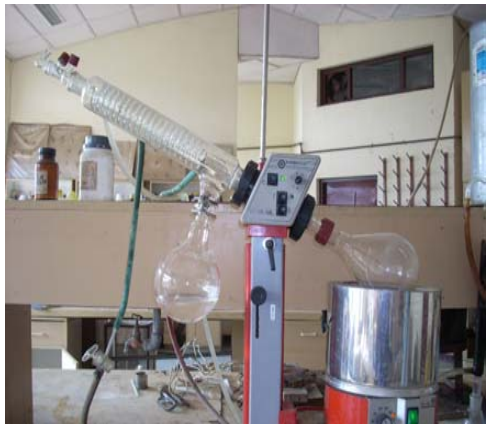

Plate 1 D: Concentration of solvent extract of *A. dubia* using rota – vacuum evaporator

Plate 1 E: Extraction of essential oil from *A. dubia* leaves

Plate 1 F: Seeds of *Parthenium hysterophorus*

Plate 2

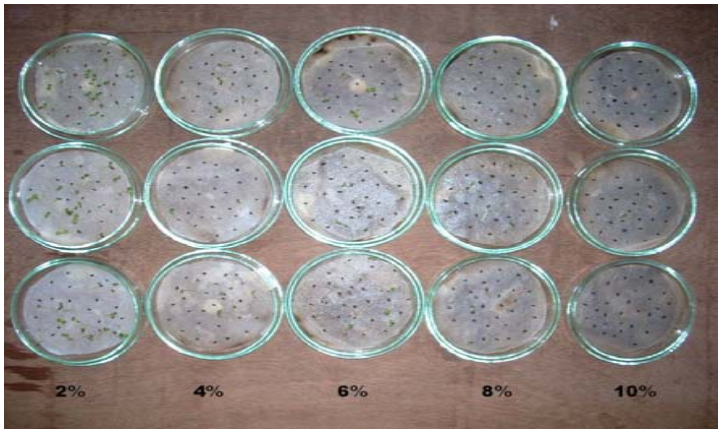

Plate 2 A: Allelopathic effect of leaf leachate extract on germination of *P. hysterophorus*

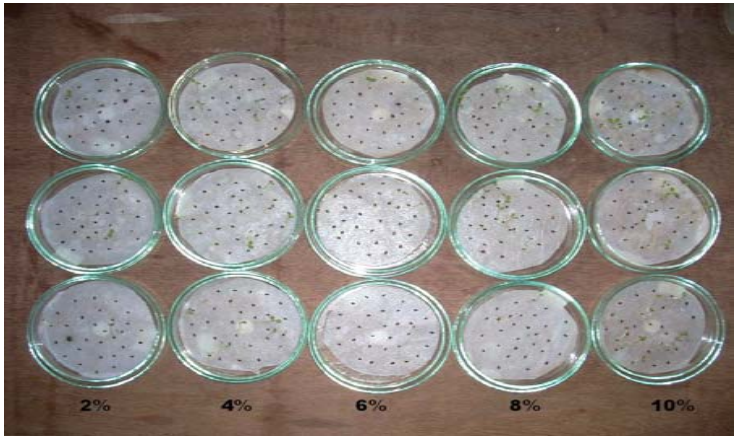

Plate 2 B: Allelopathic effect of stem leachate extract on germination of *P. hysterophorus*

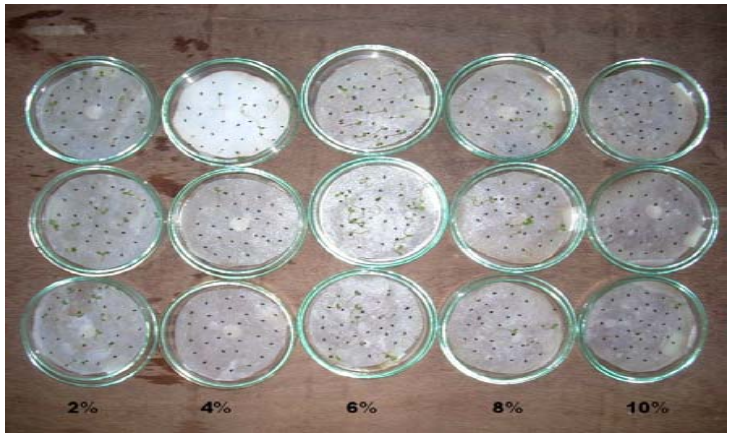

Plate 2 C: Allelopathic effect of root leachate extract on germination of *P. hysterophorus*

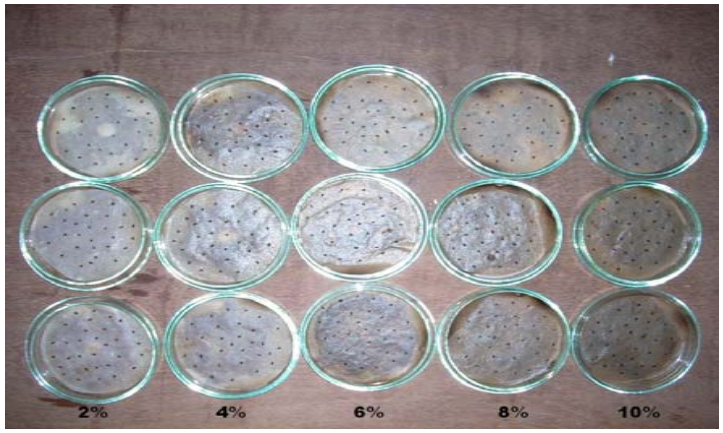

Plate 2 D: Allelopathic effect of leaf decomposed extract on germination of *P. hysterophorus*

Plate 3

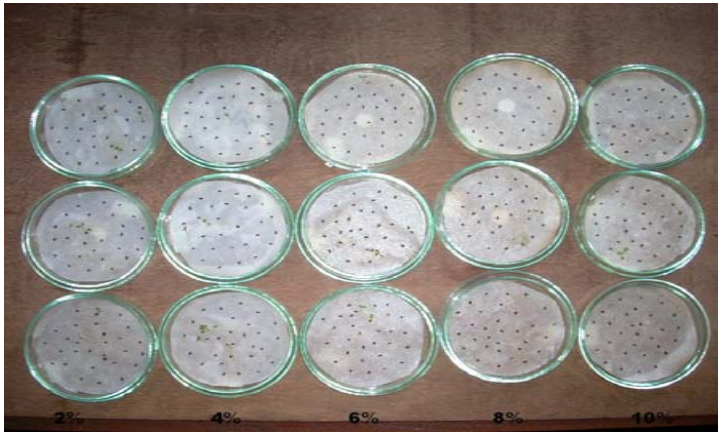

Plate 3 A: Allelopathic effect of stem decomposed extract on germination of *P. hysterophorus*

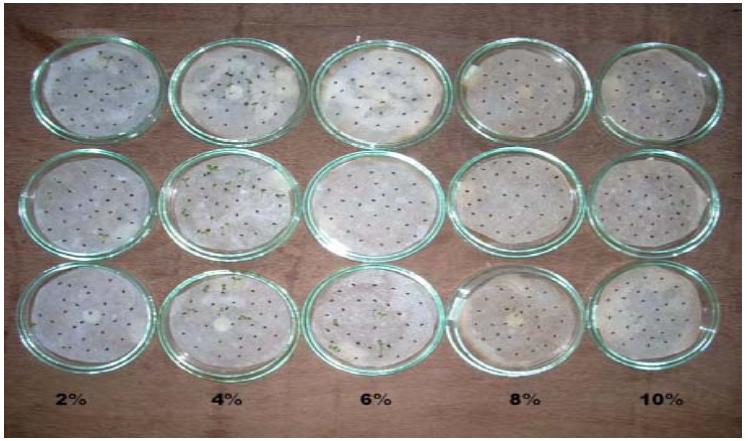

Plate 3 B: Allelopathic effect of root decomposed extract on germination of *P. hysterophorus*

Plate 3 C: Allelopathic effect of hexane leaf extract on germination of *P. hysterophorus*

Plate 3 D: Allelopathic effect of hexane stem extract on germination of *P. hysterophorus*

Plate 4

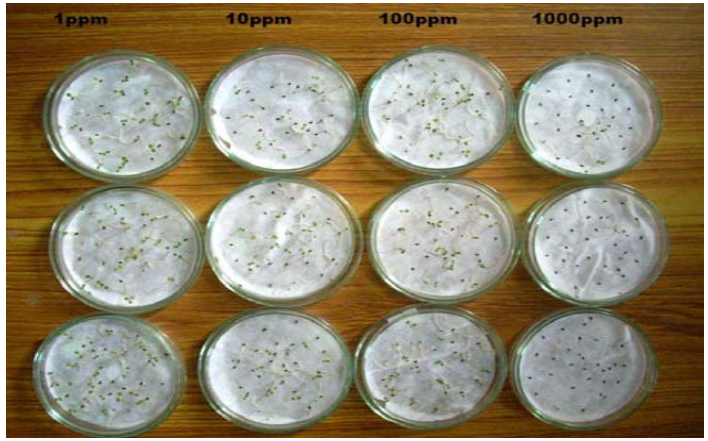

Plate 4 A: Allelopathic effect of hexane root extract on germination of *P. hysterophorus*

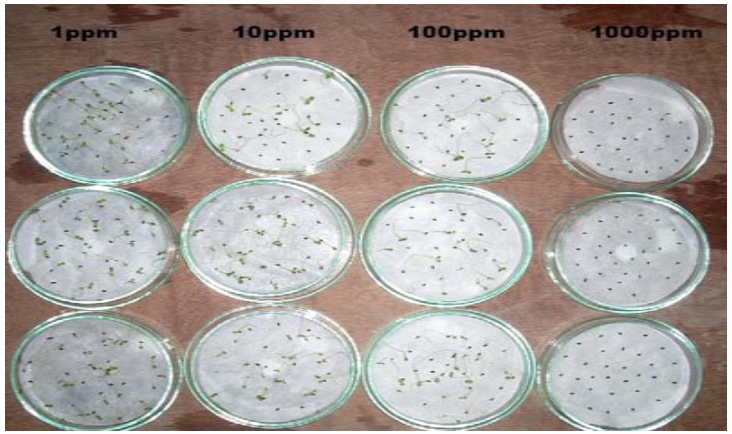

Plate 4 B: Allelopathic effect of chloroform leaf extract on germination of *P. hysterophorus*

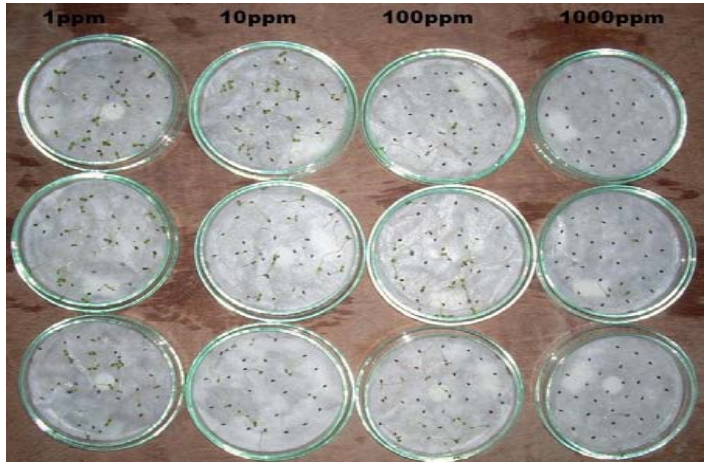

Plate 4 C: Allelopathic effect of chloroform stem extract on germination of *P. hysterophorus*

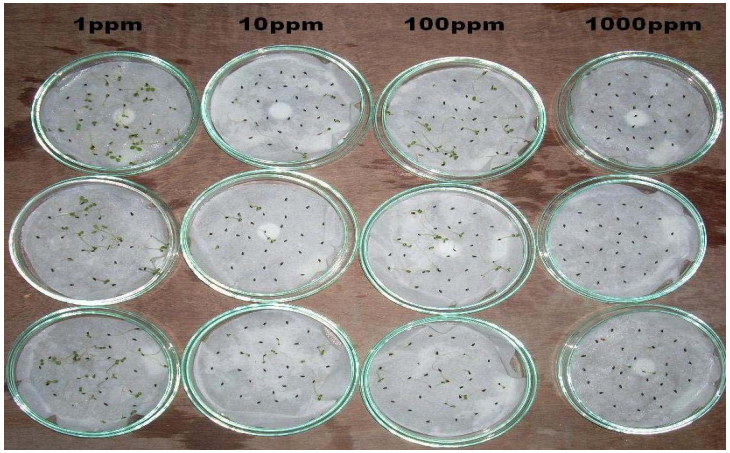

Plate 4 D: Allelopathic effect of chloroform root extract on germination of *P. hysterophorus*

Plate 5

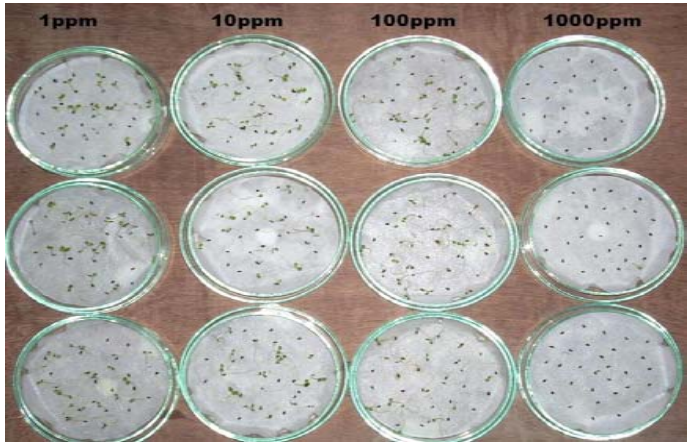

Plate 5 A: Allelopathic effect of methanol leaf extract on germination of *P. hysterophorus*

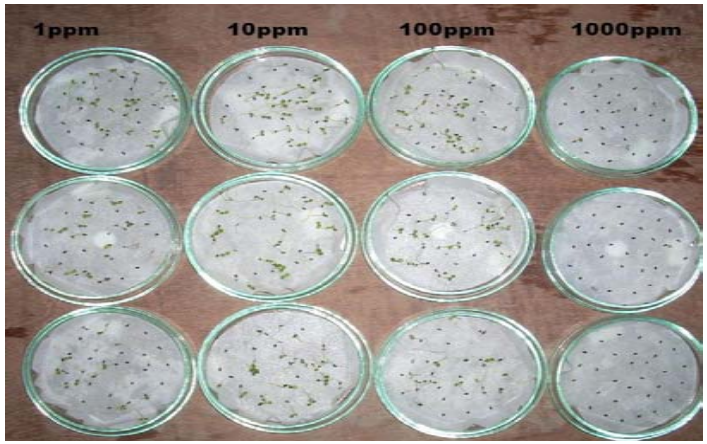

Plate 5 B: Allelopathic effect of methanol stem extract on germination of *P. hysterophorus*

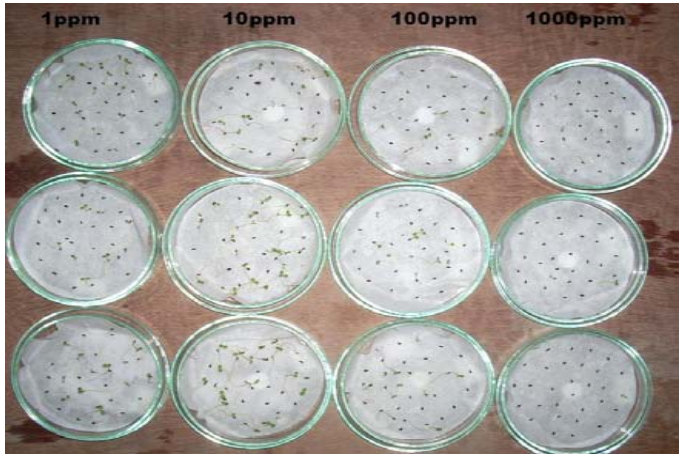

Plate 5 C: Allelopathic effect of methanol root extract on germination of *P. hysterophorus*

Plate 5 D: Allelopathic effect of aqueous leaf extract on germination of *P. hysterophorus*

Plate 6

Plate 6 A: Allelopathic effect of aqueous stem extract on germination of *P. hysterophorus*

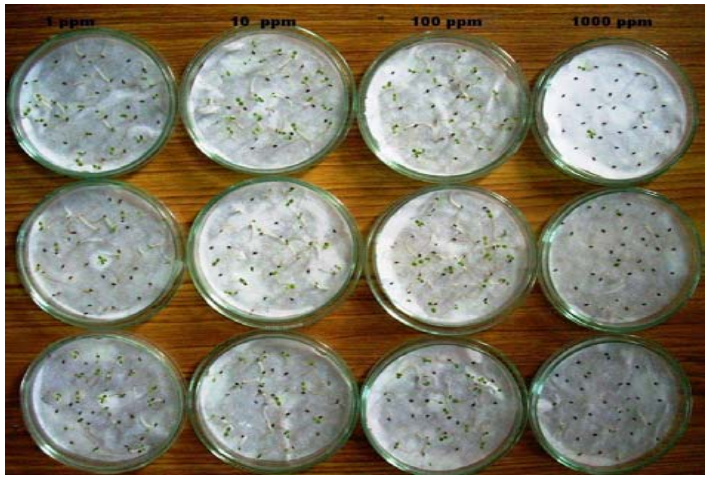

Plate 6 B: Allelopathic effect of aqueous root extract on germination of *P. hysterophorus*

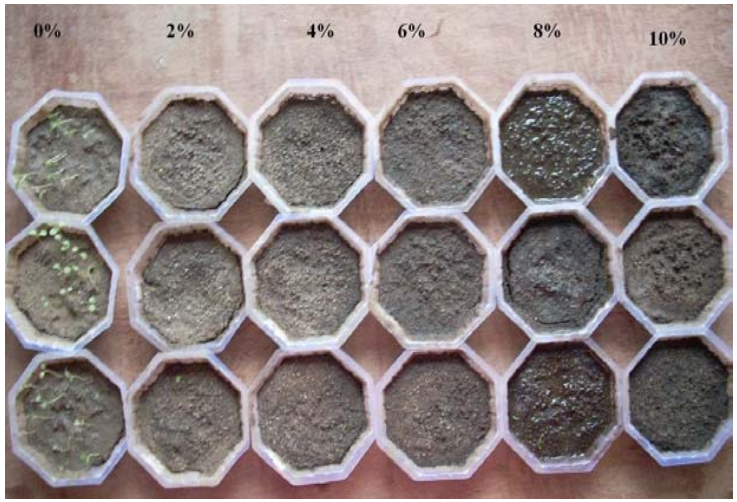

Plate 6 C: Allelopathic effect of leaf amended soil on germination of *P. hysterophorus*

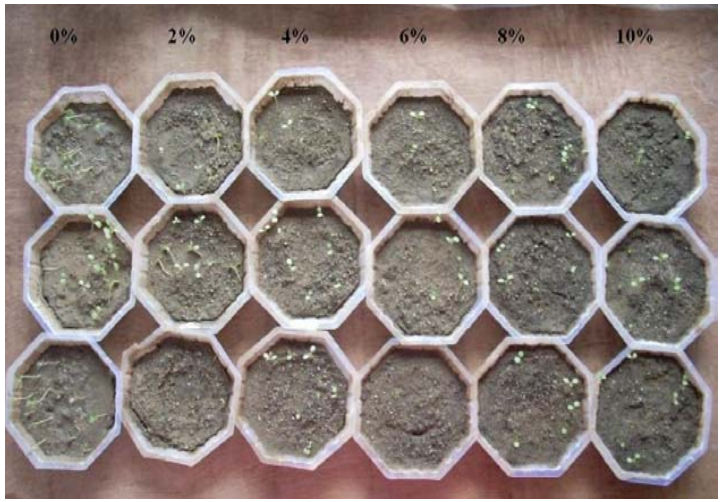

Plate 6 D: Allelopathic effect of stem amended soil on germination of *P. hysterophorus*

Plate: 7

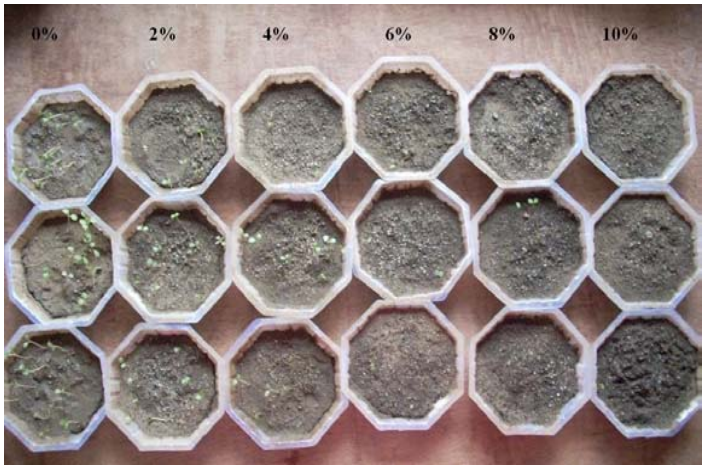

Plate 7 A: Allelopathic effect of root amended soil on germination of *P. hysterophorus*

Plate 7 B: Allelopathic effect of essential oil of *A. dubia* on germination of *P. hysterophorus*

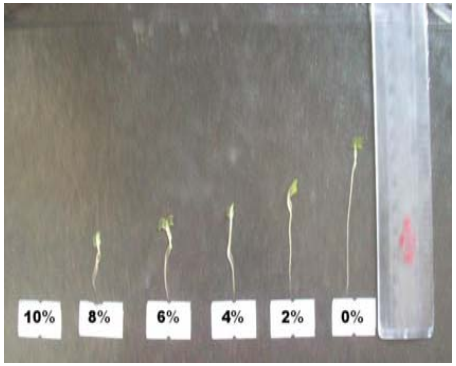

Plate 7 C: Allelopathic effect of leaf leachate extract on seedling of *P. hysterophorus*

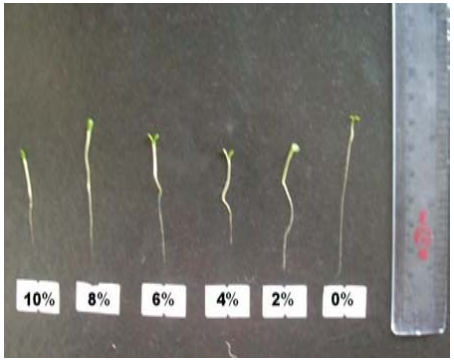

Plate 7 D: Allelopathic effect of stem leachate extract on seedling of *P. hysterophorus*

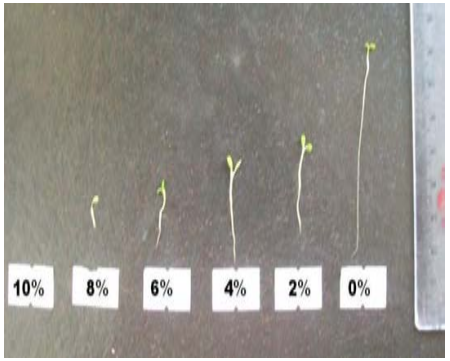

Plate 7 E: Allelopathic effect of stem decomposed extract on seedling of *P. hysterophorus*

Plate: 8

Plate 8 A: Allelopathic effect of root decomposed extract on seedling of *P. hysterophorus*

Plate 8 B: Allelopathic effect of hexane leaf extract on seedling of *P. hysterophorus*

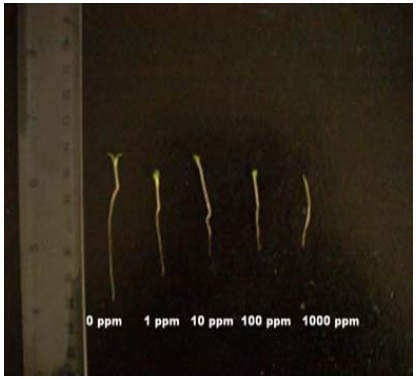

Plate 8 C: Allelopathic effect of hexane stem extract on seedling of *P. hysterophorus*

Plate 8 D: Allelopathic effect of hexane root extract on seedling of *P. hysterophorus*

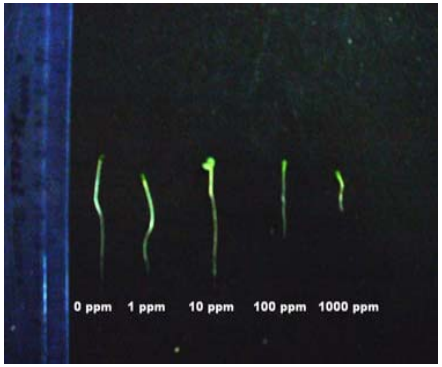

Plate 8 E: Allelopathic effect of chloroform leaf extract on seedling of *P. hysterophorus*

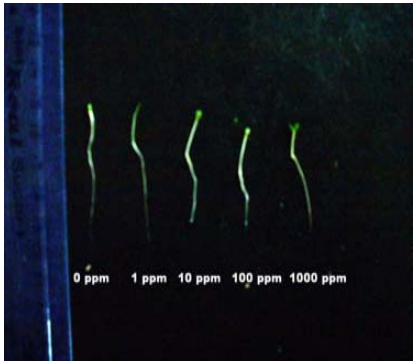

Plate 8 F: Allelopathic effect of chloroform stem extract on seedling of *P. hysterophorus*

Plate: 9

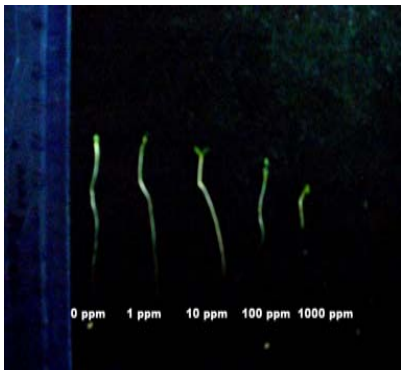

Plate 9 A: Allelopathic effect of chloroform root extract on seedling of *P. hysterophorus*

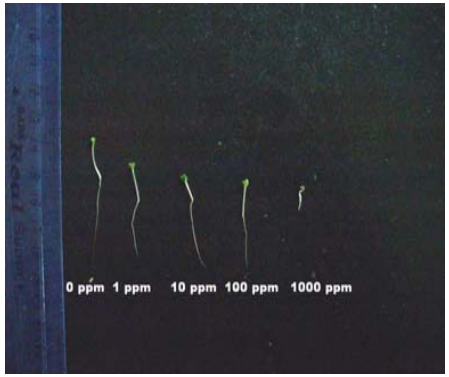

Plate 9 B: Allelopathic effect of methanol leaf extract on seedling of *P. hysterophorus*

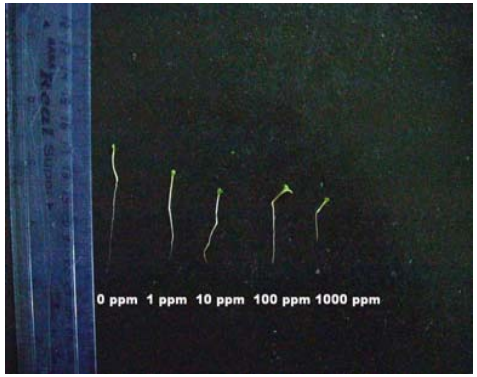

Plate 9 C: Allelopathic effect of methanol stem extract on seedling of *P. hysterophorus*

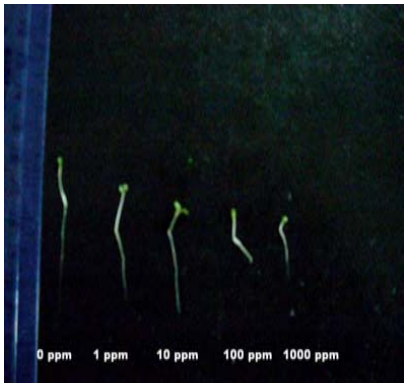

Plate 9 D: Allelopathic effect of methanol root extract on seedling of *P. hysterophorus*

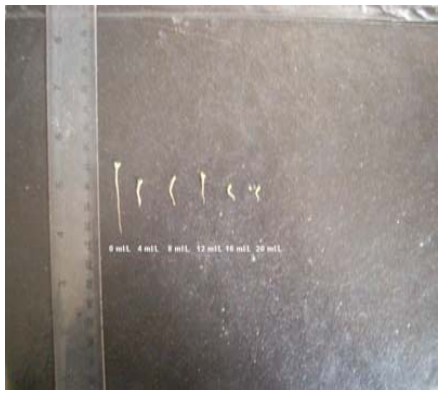

Plate 9 E: Allelopathic effect of essential oil on seedling of *P. hysterophorus*

Plate 9 F: *Parthenium* infested site and its vegetative state at top left corner.