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ALLELOPATHIC EFFECTS OF JATROPHA CURCAS L. LEAF AQUEOUS EXTRACT ON EARLY SEEDLING GROWTH OF *PARTHENIUM HYSTEROPHORUS* L.

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ABSTRACT:- *Jatropha curcas* L. (*J. curcas*) is gaining interest as biodiesel plant in Pakistan. Therefore, it is necessary to investigate the interaction of *J. curcas* not only with crop plants but also with weed species before its introduction into agroforestry systems. During present investigation, allelopathic potential of *J. curcas* leaf aqueous extract was investigated on seed germination and seedling development of *Parthenium hysterophorus* L. Extracts were applied at 25%, 20%, 15%, 10% and 5% as seed soaking for 8 hours prior to sowing. Phenolics compounds were found in aqueous extracts and were higher in 25% extract (52.72 mg gallic acid eq. / gm of extract). Higher concentration of the extract (25%) significantly decreased seed germination (%), seed germination index, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, seedling relative water content and seed vigor index. It was inferred from findings of the present investigation that aqueous extract of *J. curcas* exhibits inhibitory effects on growth and development of *P. hysterophorus* and therefore could be a potential source of botanical herbicide in the control of this noxious weed species.

Key Words: Jatropha curcas; Parthenium hysterophorus; Phenolic compounds; Allelopathy

INTRODUCTION

The process by which a plant species either stimulate or inhibit the growth and development of other neighboring plant species by pro-ducing certain chemicals that are released into the environment is referred to as allelopathy (Rice, 1984; Khattak et al., 2015). The donor plant species may affect germination, growth and development of the recipient plant species. Allelopathic compounds / allelochemicals are released into the environment by various means such as leaching, volatilization, root exudation and decomposition of the plant residue in the soil (Einhelling, 1987; Sangeetha and Baskar, 2015). Allelochemicals affect all functions of plant life including photosynthesis, respiration, transpiration, resistance and growth (Rice, 1984; Saxena et al., 2004). Allelopathy can play important role in sustainable agriculture such as pest control and weed control (Kohli et al., 1998). Recently scientists are sear-ching for biological solutions to minimize the adverse effects of synthetic herbicides and insecticides in agriculture production (Duke and Lydon, 1987). The harmful impacts of allelopathy can be exploited for the production of botanical herbicides (Sodaeizadeh et al., 2009; Sher et al., 2014).

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Weeds are the most aggressive, troublesome and undesirable element of the world's vegetation and cause reduction in crop yield, wastages of resources and human energy. *Parthenium hysterophorus* (L) belonging to family Asteraceae is an invasive weed species of wide spread in Asia, Australia and Africa (Evens, 1997; Kohli et al., 2006). It is among noxious weeds threatening crop production in different regions of the world including Pakistan. Control of Parthenium is therefore necessary in the country. It is present along road sides, footpaths, cultivated fields, water courses, and other disturbed habitats (Wiesner et al., 2007).

Jatropha curcas L. belongs to family Euphorbiaceae. It is reported that *Jatropha* seeds contain 30 to 40 % of oil (Kandpalal and Madan, 1995). *Jatropha curcas* has gained popularity as biodiesel plant in both developed and developing countries of the world. Jatropha seeds oil is non edible and is used as biodiesel energy (Achten et al., 2008). In Pakistan cultivation of *J. curcas* is expanding rapidly because of government interest in the renewable energy resources. Therefore it is necessary to evaluate interaction of *J. curcas* with weed species before its introduction into the agroforestry system. The present study investigated allelopathic effects of *J. curcas* leaf aqueous extracts on seed germination and early seedling growth of *Parthenium hyesterophorus*.

MATERIALS AND METHOD

Preparation of Aqueous Extract of *Jatropha curcas* Leaf

Fresh and fully expended leaves of *Jatropha curcas* were collected from field grown plants in the University of Science and Technology Bannu KP Pakistan; shade dried for 2 to 3 weeks and were ground finely in an electric grinder. Extracts were prepared by simple maceration technique. The leaf powder (100 g) was extracted in 200 mL autoclaved distilled water and left for 48 hr. The solution obtained was filtered to obtain the aqueous extract. The stock solution obtained was further diluted to make 25%, 20%, 15%, 10% and 5% solutions. The various solutions formed were stored at 4 °C for future use in the experiment.

Characterization of Aqueous Extracts

Total phenolics compounds analysis

Determination of total phenolics was based on the Folin-Ciocalteau method (Wolfe et al., 2003). Extract (200 µL) were mixed with freshly prepared diluted Folin-Ciocalteau reagent and 2ml of 7.5% sodium carbonate. The final mixture was diluted to 7ml with deionized water. Mixture was kept in dark at ambient condition for 2 h to complete the reaction. The absorbance was measured at 765nm using a spectrophotometer (Hitachis, U-510 Japan). The measurements were compared to a standard curve prepared by using various concentrations of gallic acid solution. The concentration of total phenolic was expressed as mg gallic acid equivalents / g of sample.

Bioassay

Seeds of *Parthenium hyesterophorus* were obtained from National Agriculture Research Center Islamabad (NARC) Pakistan. Uniform sized seeds were selected and surface

sterilized with 0.2% solution of mercuric chloride for 2-3 min followed by 3-4 times washing with double distilled water. Pot experiment (8 x 12 cm^2) was carried out in the glass house of the Department of Botany University of Science and Technology Bannu during May 2014. Pots were filled with autoclaved clay and sand (1:1). The calculated quantity (0.5 ml per seed) of *Jatropha curcas* extract was applied as seed soaking prior to sowing for 8 h to *P. hysterophorus*. The controlled treatment was made by soaking seeds of *P. hysterophorus* in autoclaved distilled water. The seeds were grown in the pots in a glass house. Pots were arranged in complete randomized design (CRD) with three replicates for each treatment.

The following treatments were made

Treatments	Concentrations
T0	Seeds of *P. hysterophorus* soaked in autoclaved distilled water
T1	Seeds of *P. hysterophorus* soaked in 5% aqueous extract of *Jatropha curcas*
T2	Seeds of *P. hysterophorus* soaked in 10% aqueous extract of *Jatropha curcas*
T3	Seeds of *P. hysterophorus* soaked in 15% aqueous extract of *Jatropha curcas*
T4	Seeds of *P. hysterophorus* soaked in 20% aqueous extract of *Jatropha curcas*
T5	Seeds of *P. hysterophorus* soaked in 25% aqueous extract of *Jatropha curcas*

The soil used as culture medium in the pots was analyzed for pH according to method of Mclean (1982). The soil used as culture medium had a pH value of 7.4.

Parameters Studied

The following growth parameters were studied.

The seed germination (%) was calculated at 10^{th} day of the experiment when no further germination took place in the control.

Seed germination (%) = (Total number of seeds germinated) /(Total number of seeds grown) x 100 .....Eq.1

Germination index = (No of seeds germinated at first count + No of seeds germinated at final count)/(Days of first count + days of final count) .....Eq2.

Seed vigor index is determined by multiplying germination percentage with seedling length of the same seed lot.

Seed Vigor Index = (Germination (%)) x (seedling length) ...Eq3.

Equation of Ellis and Roberts (1981) was used for the determination of mean germination time (MGT).

MGT = Dn/ n Eq.4

Where,

n = the number of seeds, which were germination on day D.

D = the number of days counted from the beginning of germination.

Relative water content of seedling was determined according to method of Gao (2000).

RWC (%) = [(FW-DW) / (TW-DW)] × 100 .....Eq. 5

FW = indicates fresh weight of seedlin
TW = represents turgid weight of seedling
DW = represents dry weight of seeding

The plants in all treatments were harvested 14 days after sowing. After harvest shoot length and root length were measured using a common measuring tape. Shoot fresh weight and shoot dry weight, root fresh weight and root dry weight and shoot to root ratio was determined.

Statistical Analyses

The data were analyzed statistically by analysis of variance technique (one way ANOVA). The comparison of treatment means was made by least significant differences (LSD) test (Steel and Torrie, 1984). Student Statistix (version 8.1 USA) was used for the determination of coefficient of correlation

RESULTS AND DISCUSSION

Phenolics are a group of compounds having allelopathic potential in the ecosystem. They have a hydroxyl group (-OH) which is bonded to an aromatic hydrocarbon group (Li et al., 2010). Total phenolic compounds were found higher in 25% extract (52.72 mg gallic acid eq. / gram extract) followed by 20% extract (48.51 mg gallic acid eq. / gram extract), 15% extract (41.77 mg gallic acid eq. / gram extract), 10% extract (38.62 mg gallic acid eq. / gram extract) and 5% extract (34.72 mg gallic acid eq. / gram extract) (Figure 1). In allelopathic interactions donor plant species inhibits the growth of target plant species through the production of allelochemicals providing the invader with competitive advantage (Callaway and Aschehoug, 2000; Ridenour and Callaway, 2001; Quddus et al., 2014). Phenolic compounds have significant allelopathic potential for application in agriculture as herbicides, fungicides and insecticides (Santana et al., 2009; Hussain et al., 2014).

There was significant decrease in seed germination (%) and germination index of Parthenium hysterophorus in response to higher concentration (25%) of the *J. curcas* extract. Mean germination time was not significantly affected at 25% of the extract. Shoot length was significantly increased at 5% and 10% of the extracts. (Figure 2 A, B, C, D). The inhibitory effects of higher concentrations of aqueous extracts of *J. curcas* on seed germination of Parthenium hysterophorus might be due to the presence of higher concentration of allelopathic compounds that arrested embryo growth. These results were in confirmation with those of Rizvi et al. (2000) who investigated allelopathic

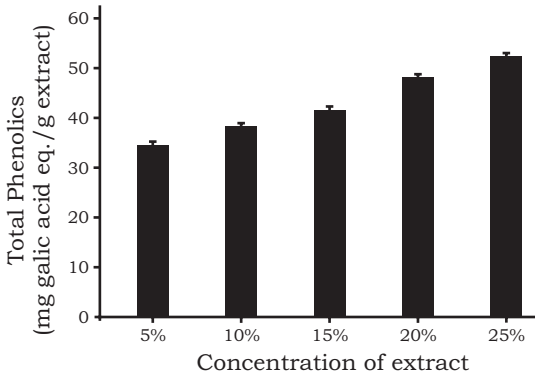
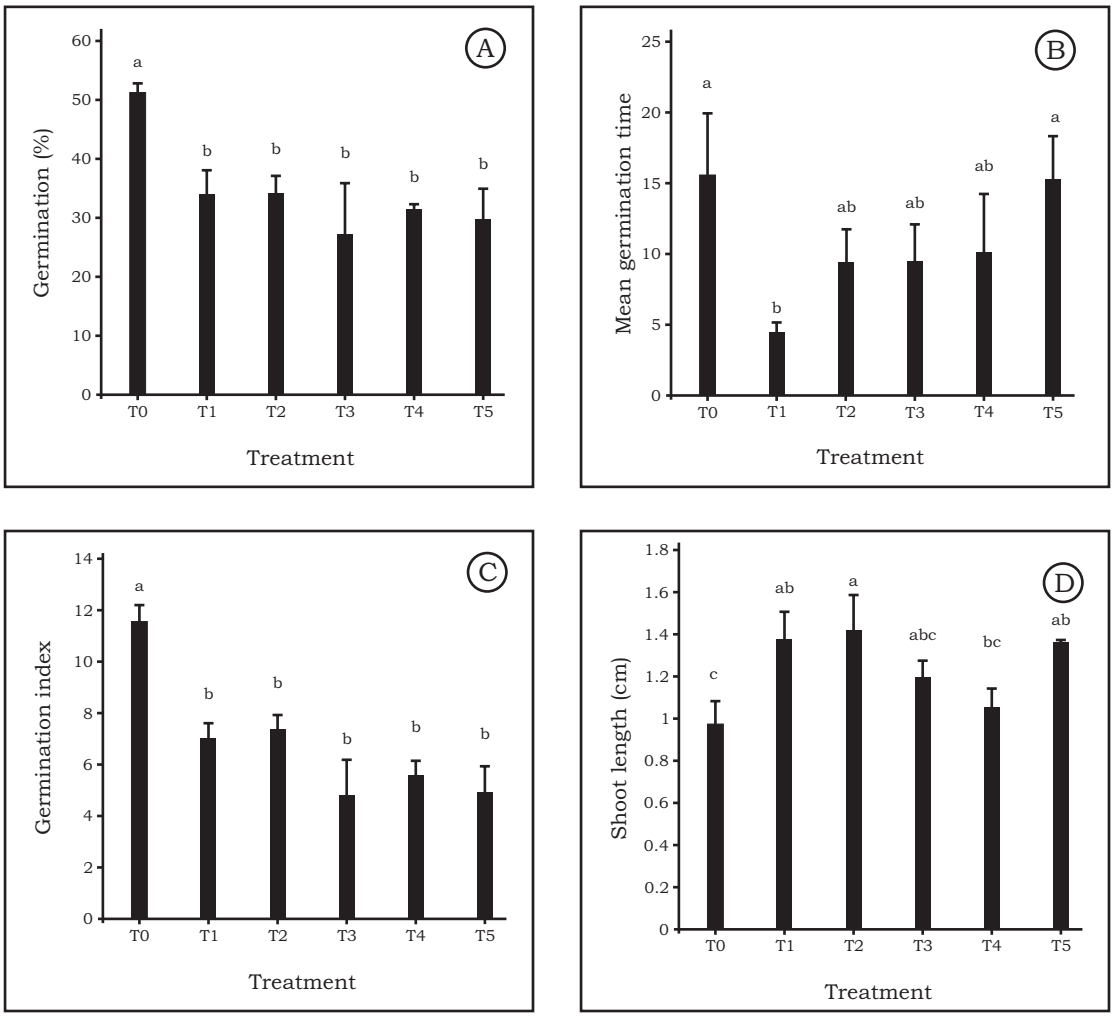


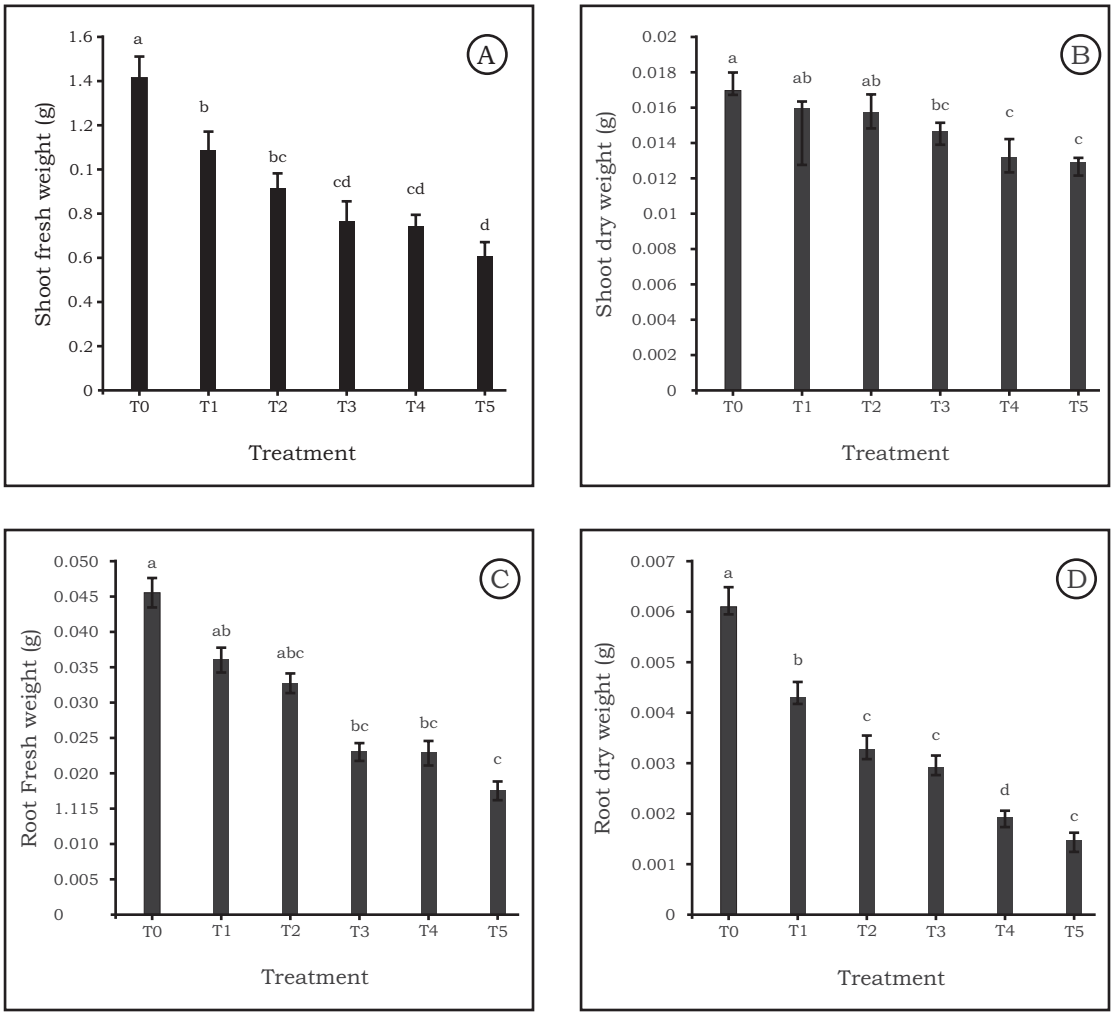
Figure 1. Total phenolics content of aqueous extracts of Jatropha curcas leaf



Treatments: T0- control, T1- 5% aqueous extract, T2-10% aqueous extract , T3-15% aqueous extract , T4- 20% aqueous extract, T5- 25% aqueous extract

Figure 2. Effect of Jatropha curcas aqueous leaf extract on (A) Germination % (B) Mean germination time (C) Germination index (D) Shoot length of Parthenium hysterophorus. Means sharing a common English letter are statistically similar

effects of wheat straw on growth of wild oat. The decrease in seed germination (%) can be correlated with the existence of phenolic compounds in aqueous extracts which prevented the action of gibberellins as they are main factors for seed germination; hence it is supposed that phenolic compounds limited performances of natural growth regulators (Saffari and Torabi-Sirchi, 2011; Knox et al., 2014). After seed germination, the effects of aqueous extracts on shoot length were stimulatory. This might be because those allelochemicals had a short lasting effect when applied as seed soaking prior to sowing. The results established that allelopathic effects of *J. curcas* leaf; in course of time decreased. These results con-

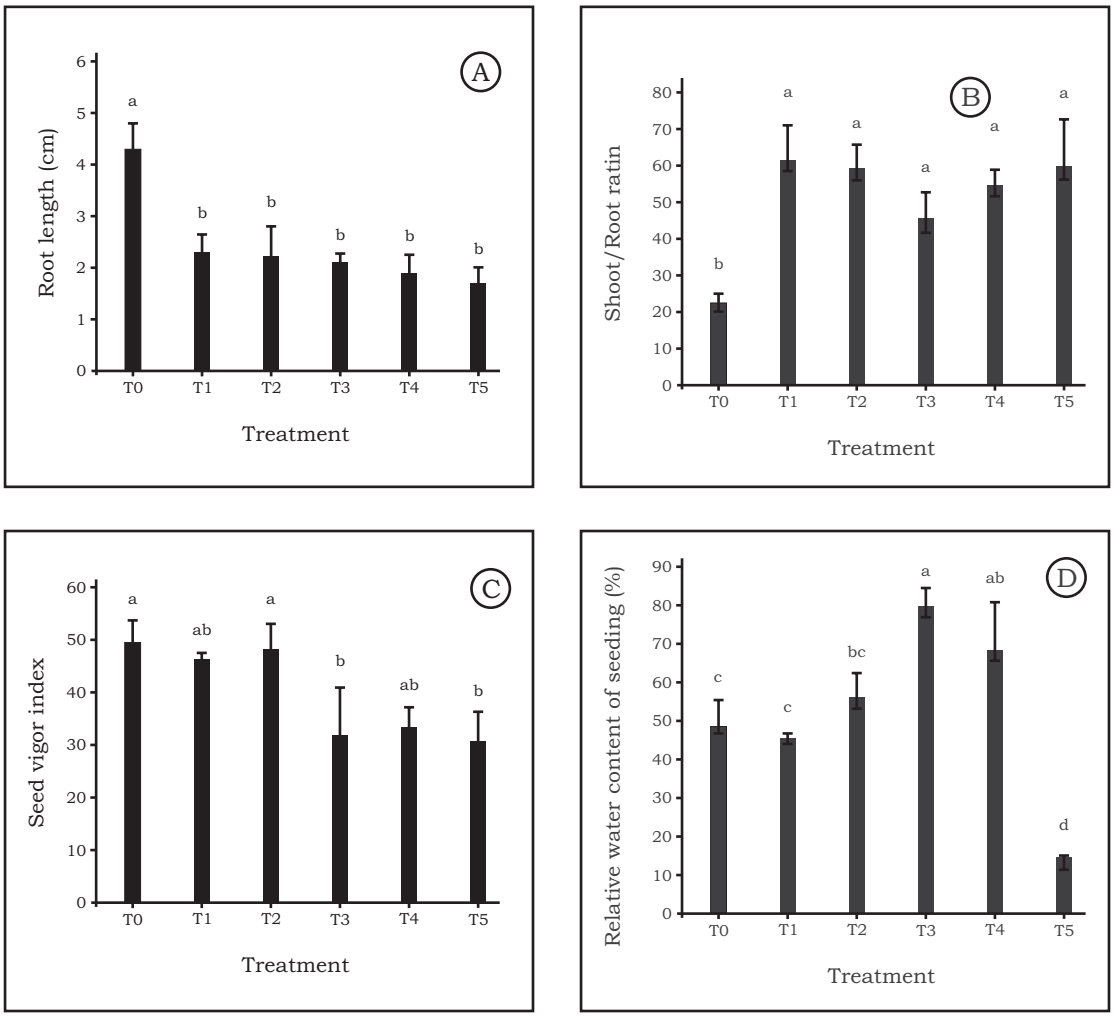


Treatments: T0- control, T1- 5% aqueous extract, T2-10% aqueous extract , T3-15% aqueous extract , T4- 20% aqueous extract, T5- 25% aqueous extract

Figure 3. Effect of Jatropha curcas aqueous leaf extract on (A) Shoot fresh weight (B) Shoot dry weight (C) Root fresh weight (D) Root dry weight of Parthenium hysterophorus. Means sharing a common English letter are statistically similar

firmed the previous findings of Prasanta and Bhowmika (2003) who have reported similar effects of different wheat straw extracts on wheat culture. The increase in shoot length can be attributed to the presence of nitrogenous and other compounds present in the extracts.

Allelochemicals suppress the mitotic activity of young cells, resulting in inhibition of seedling growth and the effect is dose dependant (Rice, 1984). Results of the present investigation showed that inhibitory effects of *J. curcas* extracts on growth attributes of Parthenium hysterophorus were concentration dependant (Figure 3 A, B, C, D). Shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were decreased by aqueous



Treatments: T0- control, T1- 5% aqueous extract, T2-10% aqueous extract , T3-15% aqueous extract , T4- 20% aqueous extract, T5- 25% aqueous extract

Figure 4 Effect of Jatropha curcas aqueous leaf extract on (A) Root length (B) Shoot / root ratio (C) Seed vigor index (D) Seedling relative water content of Parthenium hysterophorus. Means sharing a common English letter are statistically similar

extracts at all the concentrations. However, the most effective concentrations were 25% and 20% (T4 and T5).

Root length was significantly inhibited by extracts at all the concentrations with a concomitant increase in shoot / root ratio as compared to control ($p>0.05$). Seed vigor index was not significantly affected by extracts at all the concentrations. Seedling relative water content was significantly decreased (70%) by application of higher concentration (25%) of the extract as compared to control (Figure 4 A, B, C, D).

The reduction in shoot fresh weight and dry weight, root fresh weight and dry weight of *Parthenium hysterophorus* could be attributed to

the presence of phenolics compounds in aqueous extracts of *J. curcas*. Root is very sensitive indicator of phytotoxic activity. During present investigation, root growth of Parthenium hysterophorus was inhibited by aqueous extracts of *J. curcas*. Leachates, exudates and residues of a large number of plant species have been reported for allelopathy effects (Alam and Islam, 2002). Phenolic allelochemicals adversely affect root growth by inhibiting the process of mitosis in the root tips, alter cell ultra structure and then interfere with the normal growth processes of the entire plant (Cruz et al., 1998; Mondal et al., 2015). The Jatropha leaf was found as a good source of natural phenolics (Khattak et al., 2015). Batish et al. (2008) found that caffeic acid affected the growth and morphology of mong bean. Decrease in relative water content of *Parthenium hysterophorus* seedlings could be attributed to the presence of higher phenolics content in the aqueous extracts of *J. curcas* because Phenolic acids results in the induction of water stress conditions in plants (Barkosky and Einhellig, 2003).

CONCLUSION

The findings of the present investigation established that higher concentrations of the *J. curcas* leaf aqueous extracts suppressed the seed germination and growth of *Parthenium hysterophorus*. Therefore, it is inferred that *J. curcas* leaf can yield adequate amount of allelochemicals to effectively control Parthenium hysterophorus. Future studies on the isolation and purification of the effective compounds from *J. curcas* leaf could lead to the production of some novel botanical herbicides.

LITERATURE CITED

Achten, W. M. J., L. Verchot , Y. J. Franken , E. Mathijs , V. P. Singh, R. Aerts and B. Muys . 2008. Jatropha bio-diesel production and use. Biomass Bioenergy. 32: 1063–1084.

Alam, S. M. and E. U. Islam. 2002. Effect of aqueous extract of Leaf, stem and root of nettle leaf goosefoot and NaCl on germination and seedling growth of rice. Pak. J .Sci. Technol. 1: 47-52.

Barkosky, R. R. and F. A. Einhellig. 2003. Allelopathic interference of plant-water relationships by para-hydroxy benzoic acid. Bot. Bull. Acad. Sin. 44: 53-58.

Batish, D.R.M., H.P.Singh, S.Kaur, R..K. Kohli and S.S. Yadav. 2008. Caffeic acid affects earlier growth, and morphogenetic response of hypocotyl cuttings of mung bean (*Phaseolus aureus*). J. Plant. Physiol. 165: 297-305.

Callaway, R. M. and E. T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: A mechanism for exotic plant invasion. Sci. 290: 521-523.

Cruz,O.R.,A. Anaya and B. E. Hernandez-Bautista. 1998. Effects of allelochemical stress produced by sicyosdeppei on seedling root ultrastructure of Phaseolous valgaris and Cucurbita ficifolia. J. Chem. Ecol. 24: 2039-2057.

Duke, S. O. and J. Lydon .1987. Herbicide from natural compounds. Weed Technol. 1: 122-128.

Evans, H. C. 1997. *Parthenium hysterophorus*: A review of its weed status and the possibilities

for biological control. Biocont. News Inf. 18: 89-98.

Gao, F. 2000. Experimental technology in plant physiology. World Books Publishing Company, China.

Hussain, F., Barkatullah and B. Ahmad. 2014. Phytotoxic potential of Celtis *australis* L. (Family Ulmaceae) against four crop species. Pak. J. Bot .46(6): 2063-2067.

Kandpal, J. B. and M. Madan . 1995. *Jatropha curcas*: a renewable source of energy for meeting future energy needs. Renew Energy. 6: 159-160.

Khattak, A., F. Ullah, S. M. Wazir and Z.K. Shinwari. 2015. Allelopathic potential of *Jatropha curcas* L. leaf aqueous extracts on seedling growth of wheat. Pakistan. J. Bot. 47: 2449-2454.

Knox, J. A. I., D. Jaggi and M. S. Paul . 2014. Allelopathic effect of selected weeds on biochemical activity of *Parthenium hysterophorus*. Curr. Res. J. Biol. Sci. 2(4): 238-240.

Kohli, R. K., D. Batish and H. P. Singh. 1998. Allelopathy and its implication in agroecosystems. J .Crop Prod. 1: 169-202.

Kohli, R. K., D. R. Batish, H. P. Singh and K. Dogra. 2006. Status, invasiveness and environmental threats of three tropical American invasive Weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.). Biol Invasions. 8: 1501-1510.

Li, Z. H., Q. Wang, X. Ruan, C. D. Pan and D. A. Jiang. 2010. Phenolics and plant allelopathy. Molecules. 15: 8933-8952.

Mclean, E. O., R. H. Miller and D. R. Keeney. 1982. Soil pH and lime requirement. In Page, (eds.) Methods of soil analysis. Part 2 Chemical and microbiological properties.(2nd ed.). Agron. 9: 199-223.

Mondal, F. M., M. Asaduzzaman and T. Asao. 2015. Adverse effects of allelopathy from legume crops and its possible avoidance. Am J .Plant Sci. 6: 804-810.

Prasanta, C. and I. Bhowmika. 2003. Challenges and opportunities in implementing allelopathy for natural weed management. Crop Protect. 22: 661-671.

Quddus, M. S., S. M. Bellairs and P. A. S. Wurm. 2014. Acacia holosericea (Fabaceae) litter has allelopathic and physical effects on mission grass (*Cenchrus pedicellatus* and *C. polystachios*) (Poaceae) seedling establishment. Aus J. Bot. 62: 189-195.

Rice, E.L. 1984. Allelopathy, 2nd ed. Academic Press, New York.

Ridenour, W. M. and R. M. Callaway. 2001. The relative importance of allelopathy in interference: the effects of an invasive weed on a native bunchgrass. Oecol. 126: 444-450.

Rizvi, S.J.H.,V. Rizvi, M. Tahir, M.H. Rahimian, P. Shimi and A. Atri . 2000. Genetic variation in allelopathic activity of wheat (*Triticum aestivum* L.) genotypes. Wheat inf Service. 91: 25-29.

Saffari ,M.and M.H. Torabi-Sirchi 2011. Allelopathic Effects of straw extract from two native Iranian wheat varieties on the growth of two corn varieties (Single Cross 647, 704) American-Eurasian J. Agric. Environ Sci .10: 133-139.

Sangeetha, C. and P. Baskar . 2015. Allelopathy in weed manage-

ment: A critical review. Afr J. Agric Res.10: 1004-1015.

Santana, C. M., Z. S. Ferrera , M. E.T. Padrón and J. J. S. Rodríguez . 2009. Methodologies for the extraction of phenolic compounds from environmental samples: New Approaches. Molecules. 14: 298-320.

Saxena, S., K. Sharma, S. Kumar , N. K. Sand and P. B. Rao . 2004.Interference of three weed extracts on uptake of nutrient in three different varieties of paddy through radio tracer techniques. J. Environ Biol. 25: 387–393.

Christensen, S. 1993. Weed suppression in cereal varieties. Min. Agric. Statens Planeavls for sog. Denmark 104 p.

Sher, Z., F. Hussain and L. Badshah . 2014. Allelopathic potential of Mallotus philippinensis Muell. Euphorbiaceae. Pakistan .J .Bot. 46(6): 2057-61.

Sodaeizadeh, H., M. J. Rafeiolhossaini and P. H. Damme . 2009. Allelopathic activity of different plant parts of *Peganum harmala* L and identification of their growth inhibitors substance. Plant growth Regul. 59: 227-236

Wiesner, M., T. Taye , A. Hoffmann , P. Wilfried , C. Buettner , I. Mewis and C. Ulrichs . 2007. Impact of the Pan-Tropical weed *Parthenium hysterophorus* L. on human health in Ethiopia. “Utilisation of diversity in land use systems: Sustainable and organic approaches to meet human needs”, Tropentag, October 9-11, Witzenhausen.

Wolfe, K., X. Wu and R. H. Liu . 2003. Antioxidant activity of apple peels. J. Agric Food Chem. 51:609-614.