



Allelopathic effects of rhizome aqueous extract of *Cynodon dactylon* L. on seed germination and seedling growth of Legumes, Labiatae and Poaceae

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Abstract

Allelopathic effects of aqueous extract of rhizomes of *Cynodon dactylon* L. were studied on seed germination and seedling growth of three legume crops (*Phaseolus vulgaris* L., *Pisum sativum* L., and *Vicia faba* L.), three Labiatae species (*Thymus vulgaris* L., *Melissa officinalis* L., and *Mentha spicata* L.), and two species of Poaceae (*Avena fatua* L., *Sorghum halepense* L.). Seed germination of all Labiatae species was completely inhibited at treatments more than 2% rhizome extract of *Cynodon dactylon* but in other species, except *Phaseolus vulgaris*, complete failure of seed germination was recorded only at treatments more than 6% in *Vicia faba* and *Sorghum halepense* and 10% in *Pisum sativum* and *Avena fatua*. Seed germination of *Phaseolus vulgaris* was not completely inhibited but it was low at high concentration of the extract. The extract had strong inhibitory effect on root elongation of seedling in legums and Labiatae to shoot elongation in Poaceae. Rhizomes of *Cynodon dactylon* may be a source of natural herbicide against *Sorghum halepense* which will help to control invasive plants.

Keywords: *Cynodon dactylon*; Graminae; Legume crops; Labiatae; Bermudagrass; Growth

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Introduction

Allelopathy is a phenomenon by which allelopathic plants release chemical compounds into the environment through root exudation, leaching by dew and rain, and volatilization or decaying plant tissues (Rice, 1984). Allelopathy plays an important role in agroecosystems leading to a wide range of influences and interactions in biotic communities. Such influences and interactions are mainly a result of allelochemical release from the donor

plants that generally have harmful effects on the receiver plants but a selective benefit to the donor. In recent years, improved analytical methods have shown the allelopathy phenomenon in numerous species, indicating it is operative in plant ecosystems growth of competing species (Nardi et al., 2000; Watt and Weston, 2009; Mathesius and Watt, 2011). Biochemical activities of released allelochemicals are recognized worldwide by researchers and farmers because they provide an alternative to synthetic herbicides, insecticides, and nematocides in agro-ecosystems. Furthermore, allelopathy research in various aspects has been

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applied to the fields of agriculture and forestry in order to reduce environmental pollution and increase agricultural production for sustainable agriculture (Zeng et al., 2008).

Cynodon dactylon is a perennial grass, prostrate and fine-leaved, spread by stolons on the soil surface and by rhizomes underneath. It also produces seeds, but these are not important for its dispersal. It grows in the warm zones of the world (Latitude 45° N to 45° S) thus adapted to wide range of soils and climates, from rainy tropics to arid land, where it thrives in irrigated areas. The genus *Cynodon* comprises 10 species in the tropics and subtropics; *C. dactylon* is the most widespread and one of the 10 worst weeds of the world. It has numerous natural weed biotypes and its selected strains are used for pasture, lawn and turf. The *C. hirsutus* Stent and *C. plectostachyum* (Schum.) Pilger are minor weeds and pasture plants in Argentina, Australia, and Kenya. Its competitive effects are caused due to depletion of nutrients and allelopathic factors, biologically active substances produced by living and dead subterranean parts (Mahmoodzadeh, 2010). Bermudagrass produces phenolic acids and cyanogenic compounds (Ormeño-Núñez et al., 2008) and thus have allelopathic potential (Griffin et al., 2007; Li et al., 2010). Significant negative correlations were found between the growth parameters of bermudagrass and cultivated plants such as bread wheat (Yarnia, 2010b). Bermudagrass also inhibited the growth of cotton, corn and barnyardgrass (Vasilakoglou et al., 2005).

To explore allelopathic potential of *Cynodon dactylon* we examined effect of aqueous extract of rhizomes of this plant on seed germination and seedling growth of six cultivated crop species (three legumes and three Labiatae) and two wild species of Poaceae growing naturally together with *Cynodon dactylon*.

Materials and Methods

Collection of plant materials

Fresh rhizomes of *Cynodon dactylon* in its vegetative stage were collected from botany garden of Mashhad and air dried in shade for a week. The dried rhizomes were stored in plastic

bags for one month at room temperature (average during day: 25 °C) before used for experiments.

Experiments

From preliminary screening it was found that rhizomes extract had the strongest allelopathic effect on seed germination; thus we selected rhizomes for detail experiments. 10 grams of air dried rhizomes of *Cynodon dactylon* was ground, mixed with 100 ml distilled water, and left for 24 h in dark at the room temperature (average during day: 25 °C) for extraction. Aqueous extract was obtained by filtering of the mixture and final volume was adjusted to 100 ml; this gave 10% aqueous extract. The extract was considered as stock solution and a series of solution with different concentrations (2, 4, 6, and 8%) were prepared by dilution. Ten uniform and surface sterilized seeds (2% sodium hypochlorite for 15 min) of *Pisum sativum* L. were kept for germination in sterilized Petri dishes lined double with blotting paper and moistened with 10 mL of different concentrations of aqueous extracts (2 to 10%). Each treatment had three replicates (total number of test seeds was 10 x 3 = 30). One treatment was run as control with distilled water only. The Petri dishes were maintained under laboratory conditions (room temperature 25 °C at mid day, and diffused light during day) for one week. Equal volume of distilled water was added in the dishes when moisture content of the blotting paper declined. After one week, number of germinated seeds were counted and, the root and shoot length were measured. All roots and shoots from each Petri dish were cut separately and oven dried at 70 °C for 48 h to get dry biomass of root and shoot. Total biomass of seedlings was calculated as the sum of biomass of root and shoot. The same procedure was followed to evaluate allelopathic effects of *C. dactylon* on seed germination and seedling grow of other two legume crops, i.e., bean (*Phaseolus vulgaris* L.) and Broad Bean (*Vicia faba* L.); three Labiatae, namely, Thyme (*Thymus vulgaris* L.), Lemon balm (*Melissa officinalis* L.), and Mint (*Mentha spicata* L.) as well as two wild Poaceae, namely, *Avena fatua* L. and *Sorghum halepense* L.

Statistical analysis

Significance of the difference in root and shoot length of seedlings under different treatments were tested and compared using Analysis of Variance (ANOVA) and Homogeneity test. Regression analysis between treatments vs. root and shoot length of seedlings among Legumes, Labiatae, and wild Poaceae were done by compiling all data of Legumes, Labiatae, and wild Poaceae. Change in germination percentage with concentration of aqueous extract was evaluated using regression analysis for the combined data of all test species. All statistical analyses were done by SPSS (11.5).

Results

Germination

Except in *Phaseolus vulgaris*, there was complete failure of seed germination of test species in 10% aqueous extract. Even in 6% and 8% there were no germination of *Vicia faba* and *Sorghum halepense*. The inhibition of germination was found strong in the Labiatae species (*Thymus vulgaris*, *Melissa officinalis*, and *Mentha spicata*); there was no germination at >2% concentration (Fig. I). At 2% concentration, germination of *Mentha spicata* was found to be reduced more as compared to control (67.86%) followed by *Vicia faba* and *Melissa officinalis* (50%) (Fig. I). At 4% concentration, among germinating species (*Pisum sativum*, *Vicia faba*, *Phaseolus vulgaris*, *Sorghum halepense*, and *Avena fatua*) *Vicia faba* showed maximum reduction in germination (92.3%) over control.

Table 1

Analysis of Variance (ANOVA) of root and shoot length in different plant species under various treatments of rhizome aqueous extracts of *Cynodon dactylon*; df=5 for all treatments.

Plant species	parameters	F.	Sig.	Plant species	parameters	F.	Sig.
<i>Pisum sativum</i>	Root length	174.285	.000	<i>Melissa officinalis</i>	Root length	40.014	.000
	Shoot length	45.051	.000		Shoot length	165.169	.000
<i>Phaseolus vulgaris</i>	Root length	92.132	.000	<i>Mentha spicata</i>	Root length	10.594	.000
	Shoot length	39.741	.000		Shoot length	43.754	.000
<i>Vicia faba</i>	Root length	16.887	.000	<i>Avena fatua</i>	Root length	14.497	.000
	Shoot length	9.250	.000		Shoot length	28.942	.000
<i>Thymus vulgaris</i>	Root length	32.942	.000	<i>Sorghum halepense</i>	Root length	15.962	.000
	Shoot length	37.794	.000		Shoot length	20.945	.000

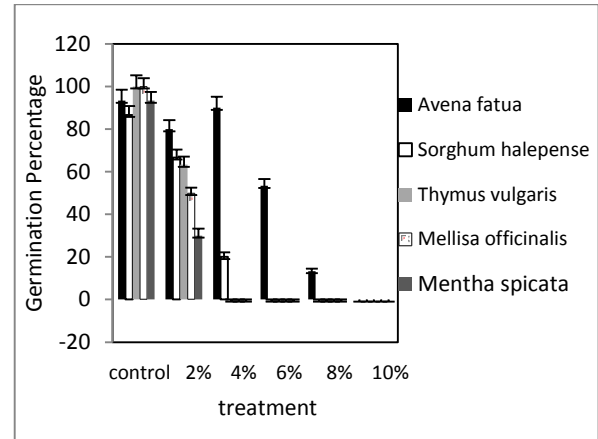


Fig. I. Seed germination percentage of studied species under different concentrations of rhizome aqueous extracts of *Cynodon dactylon*

The regression analysis between germination percentage and concentration of extract showed that 51.2% of variation in germination of the test species could be explained by concentration of rhizome extracts ($R^2 = 0.512$, Fig. II).

Seedling growth

ANOVA showed significant difference ($p < 0.05$) between treatments in root and shoot length of all test seedlings (Table 1). Among the Legumes species there was highest reduction in the root and shoot length of *Vicia faba* (Table 2). The homogeneity test showed that root length of *Pisum sativum* at treatments of 2, 4 and 6% was significantly different from that of control. The root length of *Vicia faba* at 4% and 2% was significantly different from those of control whereas in *Phaseolus vulgaris*, root length at

treatments of 4, 6, 8 and 10% was different from that of control. Among Labiatae species, the root length of *Melissa officinalis* was highly reduced (92.79%) at 2% concentration as compared to control. Among the Poaceae, the root length of *Sorghum halepense* was reduced more (70.59%) at 4% as compared to control. The root length of *Sorghum halepense* at 2% and 4% and that of *Avena fatua* at 2-8% were significantly different from that of control in both the cases (Table 2). The shoot length at 4-6% was significantly different from that of control in *Pisum sativum* (Table 2). The root length of *Vicia faba* at 2% and 4% were significantly different from that of control whereas in *Phaseolus vulgaris* it was different from control only at 6-10%. Among Labiatae species there was highest reduction in shoot length of *Mentha spicata* (80.2%) at 2% concentration (Table 2). Among Poaceae the shoot length of *Sorghum halepense* declined more (60.78%) at 4% concentration as compared to control. The shoot length of *Sorghum halepense* at 2% and that of *Avena fatua* at 6% and 8% were significantly different from that of control in both the cases (Table 3). Regression analysis between treatments vs. root and shoot length among Legumes, Labiatae, and Poaceae showed that the root and shoot length of all

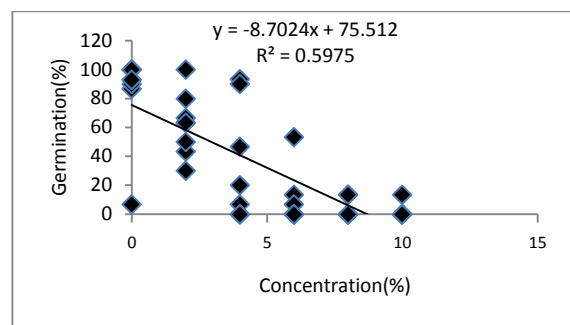


Fig. II. Regression analysis showing variation in seed germination of all test species with different concentrations of rhizome aqueous extracts of *Cynodon dactylon*

species declined with increasing concentration of the extract ($p < 0.05$) (Fig. III) but at higher concentration reduction was high for root length in the Legumes and for shoot length in Poaceae (Fig. III). In these cases high reduction was indicated by higher slope of regression line. Shoot and root biomass of seedlings of *Pisum sativum*, *Avena fatua*, and *Sorghum halepense* could not be measured because the mass was beyond the limit of our balance (0.001 g). Among the remaining species, shoot and root biomass of *Phaseolus vulgaris* seedlings were the highest (Fig. IV).

Table 2

Effect of rhizome aqueous extracts of *Cynodon dactylon* on root and shoot length of different plant species measured after one week; different letters in superscript of the values in vertical rows indicate that the values are significantly different ($p < 0.05$).

Rhizome aqueous extract concentrations	<i>Pisum sativum</i>	<i>Vicia faba</i>	<i>Phaseolus vulgaris</i>	<i>Thymus vulgaris</i>	<i>Melissa officinalis</i>	<i>Mentha spicata</i>	<i>Sorghum halepense</i>	<i>Avena fatua</i>
Root Length(cm)								
Control	8.74c	15.14c	18.54d	7.79	3.19	3.06	0.34b	0.2821c
2%	2.23b	7.38b	12.82cd	1.75	0.23	0.39	0.18a	0.21b
4%	0.38a	0.55a	10.73bc	NG	NG	NG	0.1a	0.29c
6%	0.08a	NG	4.77ab	NG	NG	NG	NG	0.11a
8%	NG	NG	0.65a	NG	NG	NG	NG	0.11a
10%	NG	NG	0.18a	NG	NG	NG	NG	NG
Shoot Length(cm)								
Control	5.43c	9.74b	16.17d	12.51	4.13	3.94	1.02c	0.7b
2%	5.26c	9.21b	10.04bcd	2.49	1.23	0.78	0.76b	0.77b
4%	2.66b	3a	10.8cd	NG	NG	NG	0.4c	0.84b
6%	0.2a	NG	2.17a	NG	NG	NG	NG	0.37a
8%	NG	NG	4.53abc	NG	NG	NG	NG	0.24a
10%	NG	NG	3.57ab	NG	NG	NG	NG	NG

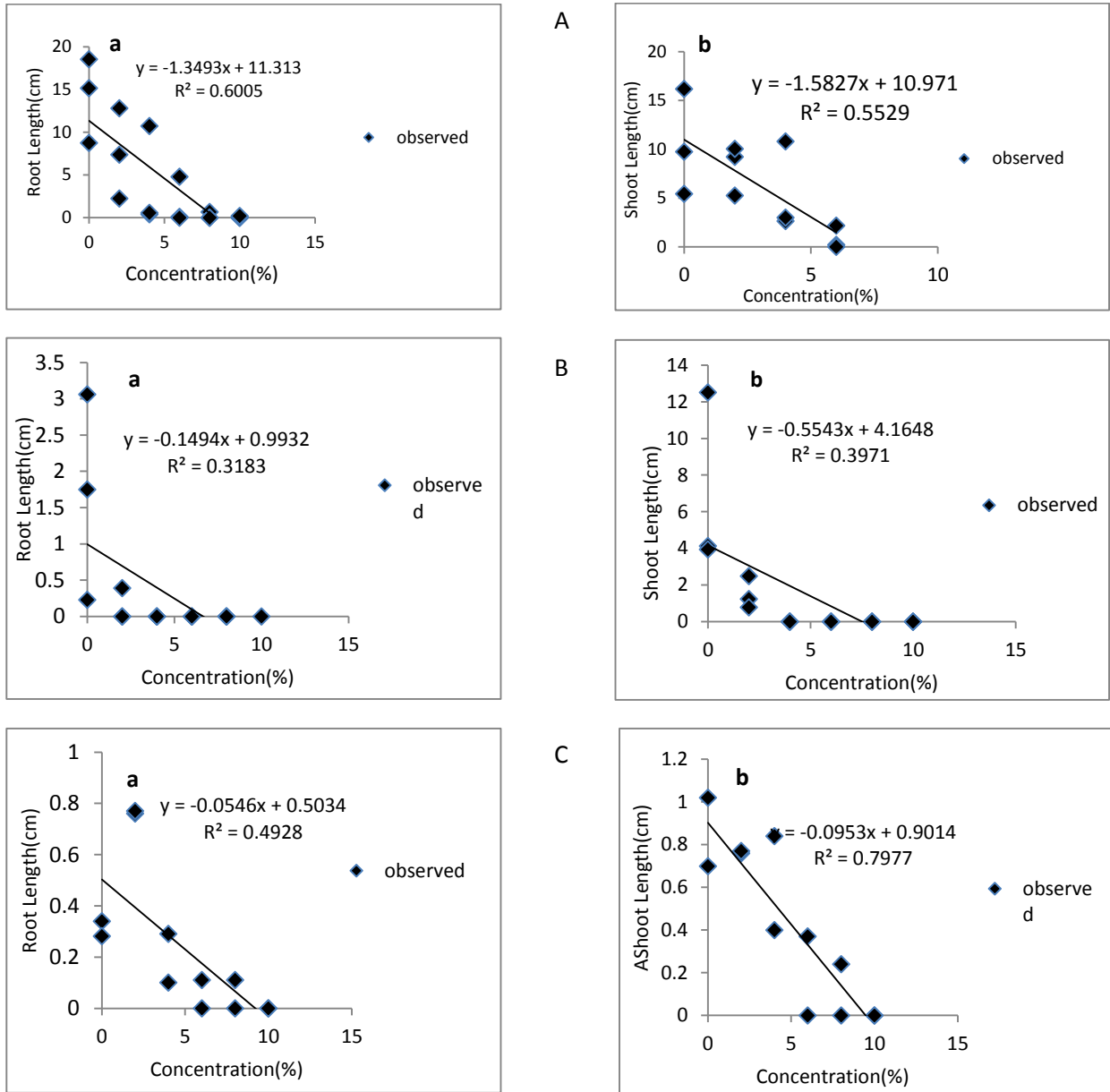


Fig. III: Relationship between different concentrations of rhizome aqueous extracts of *Cynodon dactylon* and root (a) and shoot (b) length of (A) Legume species, (B) Labiatae species and (C) Poaceae.

Discussion

From preliminary screening it was found that rhizome extract had the strongest allelopathic effect on seed germination. Mei (2005) also found that the inhibitory allelopathic impact of rhizome extract was more powerful than that of other vegetative parts. Phytochemical analysis had already shown high accumulation of growth inhibitors in rhizome of *Cynodon dactylon* (Bouchagier et al., 2008). The study demonstrated that rhizome aqueous

extracts of *Cynodon dactylon* exhibited significant inhibitory effects on seed germination and seedling growth of all test species (three Legumes, three Labiatae, and two Poaceae species) (Figs. I, II and Table 2). Earlier works have also reported that rhizome extract of *Cynodon dactylon* reduced root and shoot elongation of *Gossypium hirsutum* L. (Bouchagier et al., 2008; Bouchagier and Efthimiadis, 2010), soybeans (Verma and Rao, 2006) as well as *Triticum aestivum* (Yarnia, 2010). This indicates the availability of the inhibitory chemicals in higher

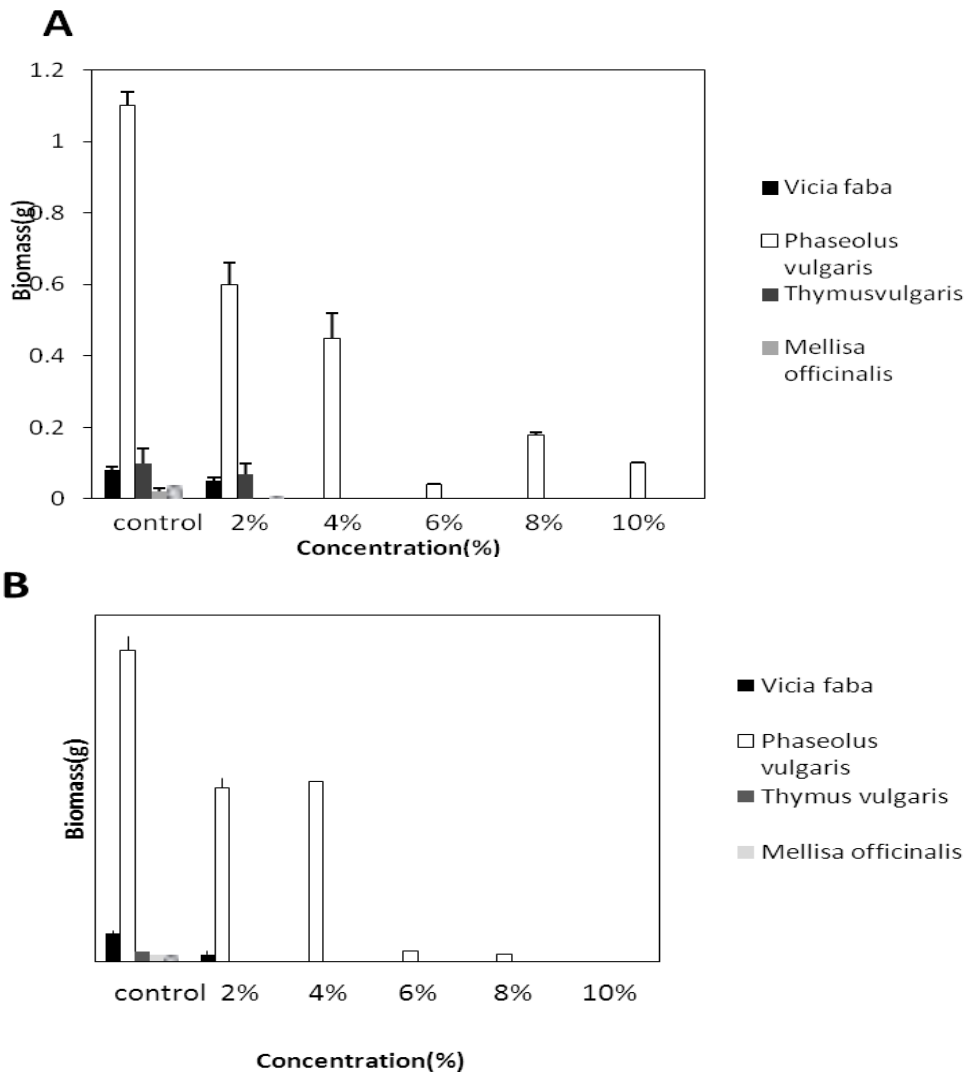


Fig. IV. Total oven dried biomass of shoot (A) and root (B) of test species seedlings in each Petri dish (10 seeds) at different concentrations.

concentration in rhizome than in stems and roots. The regression analysis between germination percentage and extract concentration showed that the germination of the test species were significantly ($p < 0.05$) reduced with the increase in the concentration ($R^2 = 0.512$, Fig. II). Among the treatments, 8% and 10% aqueous extracts had the strongest inhibitory effect on germination (Fig. I). Bouchagier et al. (2008) also reported that 10% rhizome aqueous extract of *Cynodon dactylon* resulted in complete failure of seed germination in *Gossypium hirsutum*. Vasilakoglou et al. (2005) also found a strong positive correlation between extract concentration of rhizome of *Cynodon dactylon* and reduction in seedling length of cotton, corn and barnyardgrass. The 10%

rhizome aqueous extract completely inhibited seed germination of *Pisum sativum*, *Vicia faba*, *Sorghum halepense*, *Avena fatua*, *Thymus vulgaris*, *Melissa officinalis*, and *Mentha spicata*. This could occur only when some allelochemicals present in the rhizome extract prevented growth of embryo, or caused the death. The extract of many allelopathic plants induced a variety of chromosomal aberrations in dividing cells, which increased significantly with increasing concentrations and durations of exposure (Rajendiran, 2005). At 4% concentration, among germinating species (*Pisum sativum*, *Vicia faba*, *Phaseolus vulgaris*, *Sorghum halepense*, and *Avena fatua*), the *Vicia faba* showed maximum reduction in germination (92.3%) over control. A reduction in seed germination of legumes by 80-

90% due to treating their seeds with rhizome aqueous extract of *Cynodon dactylon* has been reported by Vasilakoglou et al. (2005). In the present study there was strong inhibition in seed germination of the Labiatae species (*Thymus vulgaris*, *Melissa officinalis*, and *Mentha spicata*) even at 2% concentration and above this level complete inhibition was observed (Fig. 1).

The seeds of Labiatae appeared to be the most sensitive among the test species to inhibitory effect of rhizome aqueous extract of *Cynodon dactylon*. From the homogeneity test it was found that the shoot length in all test species except *Sorghum halepense* at 2% concentration was not significantly different from that of control whereas at the same concentration (2%) root length (except in *Phaseolus vulgaris*) was significantly different from the control (Table 2). This indicates that root elongation was affected more than that of the shoot. Similar effect of rhizome aqueous extract of *Cynodon dactylon* was reported by Vasilakoglou et al. (2005) on cotton, corn and barnyard grass. The strong inhibitory effects that *Cynodon dactylon* had on root elongation might be due to direct contact of root with the extract (Quasem, 2001). At higher concentrations reduction in length was higher for root than shoot in Legumes and Labiatae (higher slope of regression line for RL, Fig. III a-b); for Poaceae, reduction in shoot length was higher than in root length at higher concentrations (Fig. IIIc). Thus, sensitivity to allelochemicals and extent of inhibition varied with species and organs of the test species. The inhibitory effect of *Cynodon dactylon* on seed germination and seedling growth of different plant species is due to the presence of growth inhibitors (allelochemicals) in the extracts. Vasilakoglou et al. (2005) reported the presence of plant growth inhibitors in *Cynodon dactylon*. This plant releases a number of water soluble allelochemicals such as phenolic acid, ferulic, p-cumaric, vanillic, and caffeic acids. Phenolics found in rhizomes have inhibitory effects on growth of nitrogen fixing and nitrifying bacteria (Vasilakoglou et al., 2005). According to Rice (1984) phenolics are the most common and widely distributed water soluble allelochemicals. The escape of these chemicals into the environment occurs through various mechanisms

such as leaching, volatilization, and microbial decay of dead and fallen parts, as well as root exudation (Rice, 1984). These chemicals were reported to have had allelopathic potential on various agronomic crops and weeds (Li et al., 2010). Cao (2010) isolated 9 phenolic acids, namely, protocatechuic, caffeic, salicylic, syringic, ferulic, vanillic, p-coumaric, cinnamonic, and p-hydroxy benzoic acids from the rhizome aqueous extracts of *Cynodon dactylon* by high performance liquid chromatography (HPLC) and demonstrated that these compounds significantly decreased germination of ryegrass, common crabgrass, and goose grass seeds and adversely affected seedling growth.

Present results showed that concentrated aqueous extract of rhizome of *Cynodon dactylon* inhibited seed germination and seedling growth of other weeds such as *Sorghum halepense* and *Avena fatua*, the former being another invasive plant of the region. This result is also supported by the study reported by Cao (2010). He reported germination inhibition of *Lolium multiflorum*, *Poa annua*, *Digitaria sanguinalis*, *Echinochloa crusgalli*, and *Eleusine indica* by *Cynodon dactylon*. Thus, this plant could be exploited as a source of natural herbicides by maximizing its use for future weed management programmers.

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