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HERBICIDES EFFECTIVENESS AGAINST ROOT ROT FUNGI AND GROWTH OF CROP PLANTS

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ABSTRACT

Herbicides such as bromacil and bromoxynil were used to study the activity against root infecting pathogens namely; *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. Herbicides were purchased from Agrochemical market of Karachi and applied as soil drenching (0.1, 1.0 and 2% v/v concentration) in pots and observe its effect on growth of cowpea and mung bean plants. Both herbicides gave significant effect on tested crops growth in contrast to control. Highest shoot and root weight of mung bean and cowpea were recorded when 2% (v/v) concentration of bromoxynil was used. Maximum suppression in colonization of root infecting fungi was recorded at 2% (v/v) concentration by both herbicides followed by 1.0 and 0.1% (v/v) concentrations. Out of all three concentrations tested, soil drenching with 2% (v/v) by both herbicides showed effective results which was selected for the field experiment. In field experiment, shoot length and weight were increased and also showed a greater number of nodules in mung bean and cowpea plants as compared to control. It was interesting to note that the weeds were found on the control plants, while it showed complete suppression in the treated plots with both herbicides. *Fusarium* spp., *R. solani* and *M. phaseolina* colonization were significantly reduced when soil was drenched with bromacil followed by bromoxynil herbicide.

Keywords: Bromoxynil, mung bean and cowpea, root infecting fungi, soil drenching.

INTRODUCTION

Herbicides (weed killers) were first time discovered in second World War, also called as 2, 4-Dichlorophenoxyacetic acid (2**,** 4-D) used as biochemical agents (Andrew *et al*., 2011). Herbicide concentration and its effectiveness on agricultural field have been depending on the application rates and the type of herbicide used (Sebiomo *et al*., 2011). However, its effects on soil, either increasing or decreasing, depend on the chemicals type and concentration on microbial species and environmental conditions (Zain *et al*., 2013). Herbicides such as bromacil (5-Bromo-3sec-butyl-6-methyluracil) is an effective herbicide used to control perennial, annual, broad leaf and grasses (Gardiner *et al.*, 1969) while, bromoxynil (3, 5-dibromo-4-hydroxybenzonitrile) is especially effective in the control of weeds reported in cereal, corn, sorghum, onions, flax, mint, turf and on non-crop land (Cupples *et al*., 2005). Herbicides produce both positive as well as negative effect on soil microbial microorganisms and their biomass depending on the concentration and its type (Scholter *et al.* 2003). Perucci *et al.* (2000) reported that rimsulfuron and imazethapyr herbicide when applied at field rates produce harmful effect on biochemical properties of soil and also on soil microbes. Sulfonyl urea herbicide decrease the amino acid assimilation ability of some bacteria which leads to declination of microbial biomass (Allievi and Gigliotti, 2001). Similarly several scientist reported toxic effect of bromoxynil herbicide on soil microbes in their research (El-Ghamry *et al.* 2000; Pampulha and Oliveira, 2006). Reduced population of nitrifying bacteria and inhibiting activity of ammonium oxidizing bacteria due to application of bromoxynil herbicide in soil was reported by Ratnayak and Audus (1978); Pampulha and Oliveira (2006).

Root rot pathogenic fungi attack on roots limiting nutritional uptake resulting in the death of plants. Some common root infecting fungi present in Pakistan soil includes *Alternaria alternata, A. tenuissima, Botryodiplodia theobromae, Curvularia clavata, C. lunata, Drechslera australiensis, D. hawaiiensis, Fusarium moniliforme, F. oxysporum, F. semitectum, F. solani, Macrophomina phaseolina* and *Rhizoctonia solani* (Ghaffar, 1998). Of these, *Fusarium* spp. produces various symptoms namely wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting and damping-off diseases in various crops, including tomato, tobacco, legumes, cucurbits, sweet potatoes and banana and some other herbaceous plants (Synder and Hansen, 1940; Nelson *et al*., 1983). Similarly, *M. phaseolina* (charcoal rot fungus) and *R. solani* (foot rot and root rot fungi) present in soil in the form of mycelium and produces symptoms on various hosts (Sinclair, 1982; Mirza and Qureshi, 1978; Shahzad *et al*., 1988; Parmeter, 1970). *R. solani* attack plants at any stage of growth and produces symptoms like reddish brown to brown lesions, seedling damping off, girdled stem causing severe stunting and yellowing and thread like filamentous mycelium of fungus was easily observed with a hand lens (Ernest, 2013). *Macrophomina phaseolina* is a soil borne facultative parasite whose growth favored by high temperature of 30-35ºC, moisture stress, plant injury, flowering and seedling injury (Canaday *et al.,* 1986; Magalhaes *et al.,* 1982; Manici *et al.,* 1995; Pearson *et al.,* 1984). It survives as sclerotium in soil or inside diseased plant tissues.

Herbicides application in soil may lead to adsorption on clay minerals, organic matter of soil, organoclay complexes which results in increment of herbicide concentrations on top layer of soil, becoming dangerous for other seasonal crops (Majka and Lavy, 1977; Sanchez-Camazano *et al.,* 2000; El-Nahhal *et al.,* 1998; 1999; Thurman *et al.,* 1996). For this purpose, applied herbicide having some active ingredients and helpful in balanced ecosystem. For this reason, experiments were conducted on herbicides application in the improvement of crops growth and reduction of soil borne pathogenic fungi.

MATERIALS AND METHODS

Preparation of herbicides

Two herbicides such as Bromacil and Bromoxynil were purchased from Agrochemical market of Karachi in the form of concentrated liquid. The stock solution is prepared by adding 250mL of sterilized water in 100mL concentrated solution of both herbicides separately. Further concentrations were made by adding sterile distilled water in the stock solutions.

Physical properties of soil

Pots were filled with 300 g of soil, obtained from experimental plot of Department of Botany, University of Karachi. Soil was examined for their physical properties like nature (sandy loam), pH (7-7.6), moisture holding capacity (24.04%), total organic matter (24%) (Keen and Raczkowski, 1922). Natural population of soil borne fungi were also estimated using different techniques like wet sieving technique for *M. phaseolina* (0-3 sclerotia/g), baiting technique for isolation of *R. solani* (6-10%) and for observing *Fusarium* spp., soil dilution technique (3700 CFU/g) were used (Sheikh and Ghaffar, 1975; Wilhelm, 1955; Nash and Snyder, 1962).

Pot experiments

Four treatments (soil drenching with each herbicide at 0, 0.1, 1 and 2 % [v/v] respectively) with 3 replicates of each treatment were placed in randomized manner on screen house bench of Department of Botany, University of Karachi. Four seeds of each crop (mung bean and cowpea) were sown in pot which on germination thinned to two plants per pot. 0% treatment containing soil drenching with 4 mL of distilled water (served as control). Before sowing all seeds were surface sterilized with 0.1% Calcium hypochlorite, washed thrice with distilled water and dried aseptically. Thirty days after germination of plants, experiment was terminated and different growth parameters were recorded.

Field experiments

Small size plots of 2x2 were prepared in the field of Department of Botany, University of Karachi. Soil drenching with 2% concentration of both herbicides was selected in field. Eight seeds of mung bean and cowpea were sown in each plot which after germination thinned to four seedlings. Data of growth and infection in roots were recorded after one month of germination.

Observation of root infecting fungi

Mung bean and cowpea roots were cut into 5 pieces of 1 cm and after surface sterilization with 1% calcium hypochlorite, were placed on Petri plates having Potato Dextrose Agar containing antibiotic (penicillin @ 100,000/liter and streptomycin @ 20mg/L). Incubate these Petri plates at room temperature (27-33°C) for one week to record colonization of roots by pathogenic root infecting fungi (Kanwal *et al.,* 2017; Rafi *et al.,* 2016).

Data analysis

All experiments were performed in a replicate of three and averages of growth parameters and colonization by root infecting fungi were calculated and run on COSTAT program and Duncan’s Multiple Range Test were also performed according to Sokal and Rohlf (1995).

RESULTS

Pot experiment: Plant height was significantly (P<0.05) increased when soil was drenched with 1% (v/v) bromacil but highest shoot length, root length and root weight were recorded when 2% (v/v) concentration of bromoxynil was used. Bromacil and bromoxynil impressively reduced *Fusarium* spp. colonization when soil drenching was carried out with 2% concentration (P<0.05). The frequency of colonization decreases as the concentrations of herbicides increases. *R. solani* colonization was reduced when soil was drenched with 2 % of both herbicides in cowpea while 1 and 2 % v/v of bromoxynil reduced *R. solani* colonization in mung bean plant. In case of *M. phaseolina*, colonization % was decreased when soil was drenched with 1% concentration of bromacil followed by 2% concentration of bromoxynil (Fig. 1).

Both herbicides bromacil and bromoxynil were equally effective at 2 % concentration followed by 1 % concentration in improvement of growth and control of root rot fungi of crop plants.

Field experiment: In mung bean, shoot length was significantly increased (P<0.05) when soil was drenched with bromacil and bomoxynil as compared to control. In comparison between bromacil and bromoxynil, bromacil showed greater enhancement (P<0.05) in shoot length while shoot weight significantly increased (P<0.05) with bromoxynil. No affects was recorded on a number of leaves while number of nodules were enhanced with bromoxynil. Significant (P<0.05) results were observed in controlling the weeds. Two types of weeds were recorded in control plots while both herbicides such as bromacil and bromoxynil controlled the weeds in treated plots. The colonization percentage of root infecting fungi such as *Fusarium* spp., *R. solani* and *M. phaseolina* showed maximum inhibition when soil was drenched with bromacil and bromoxynil herbicides (Fig. 2). Herbicides showed greater effect on the growth parameters of cowpea. Shoot length increased with the use of bromacil and bromoxynil while shoot weight significantly (P<0.01) increased with the use of bromoxynil which showed that in the presence of herbicides cowpea plants become healthy while reduction in root rot fungi was recorded by the use of both herbicides. Number of nodules and number of leaves increased, when the soil was drenched with herbicides such as bromacil and bromoxynil. Both the herbicides also reduced the number of weeds in treated plots as compared to the control plants.

Out of two herbicides used in the field experiment, bromoxynil gave the best effect on plant health and also in the control of root rot fungi.

DISCUSSION

Effect of bromacil and bromoxynil as soil drenching was observed for their effectiveness on plant growth and controlling root pathogens. Generally herbicides used for control of unwanted plants or weeds. According to Mussa and Russel (1977), herbicides can transform the host plant structure and defense mechanisms results in enhancement of susceptibility to infection by stimulating or inhibition of associated microbes (Smiley and Wilkins, 1992). Bromacil and bromoxynil impressively reduced *Fusarium* spp. when soil drenching was carried out with 2% concentration of both the herbicides whereas colonization percentage of *Macrophomina phaseolina* growth decreased when soil was drenched with 1% concentration of bromacil and 2% concentration of bromoxynil. It was reported that bromoxynil herbicide caused suppressive effect in microbial activity and biomass by producing toxic effect on them (Abbas *et al.*, 2014). Most of the researcher showed that by using herbicides on trees and other ornamental plants, growth parameters gradually increased (Davison, 1983). Roots are essential for absorbing water and mineral uptake and are predominantly found beyond the drip line. The studies on root system showed increment in root length and weight when herbicides are applied (Schnelle *et al*., 1989). According to Davies (1987), when plots were treated with herbicides, growth of plants was better than in cultivated plants. However, when management skills are low and non-selective herbicides such as imazapyr was used, it may cause severe damaged on plants (Ivens, 1996). The important part of herbicide is to produce positive impact on the crop plants in the presence of herbicide. Using a higher concentration of herbicides in treatment, results of microbial count were much lower when compared to soil treated with recommended doses (Ayansina and Oso, 2006). Experiments have shown that microbes may use herbicides as a source of carbon. It increased the microbial populations as the time period increases (Radosevich *et al.*, 1995). Field experiment showed that when herbicides used as soil drenching, it increased growth parameters on crop plants. Shoot length and weight were increased when bromoxynil herbicides used. Greater number of nodules were recorded when both bromacil and bromoxynil herbicides used as compared to control.

Cowpea **Mung bean**

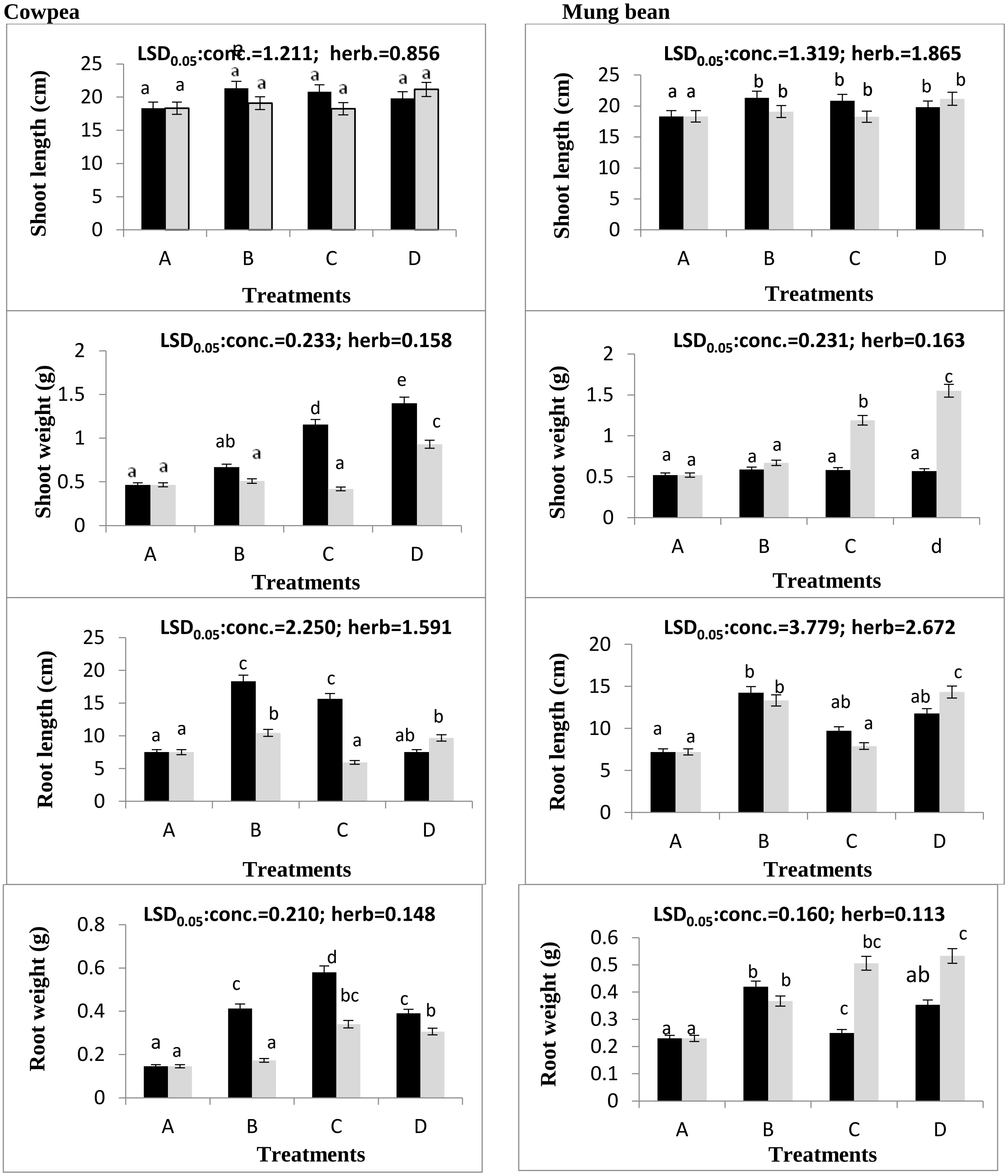

Figure 1 continued.....

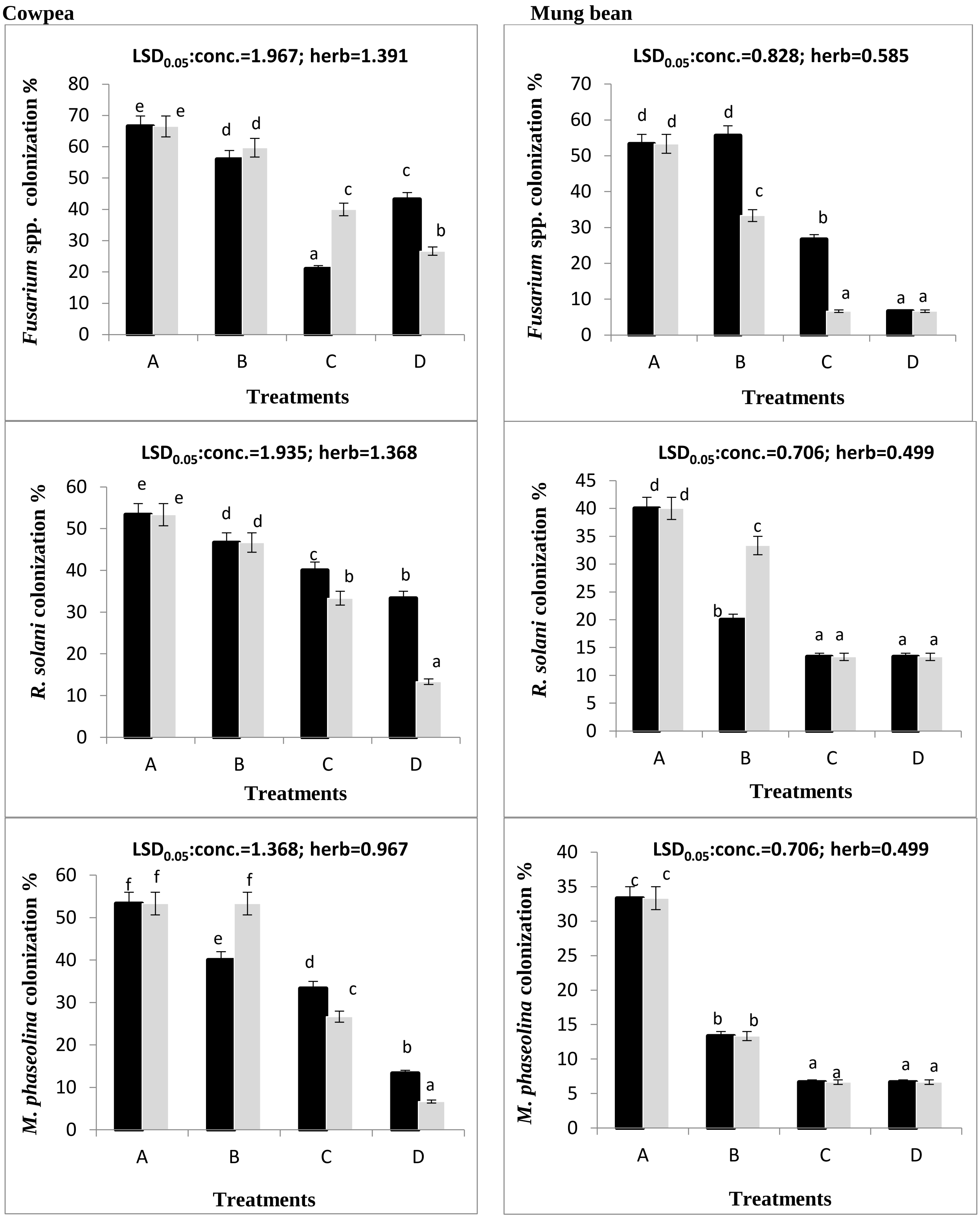

Fig. 1. Soil drenching with herbicides on growth parameters and control of root rot fungi on mung bean and cowpea plants.

Herbicide 1= Bromacil Herbicide 2= Bromoxynil

Where, **A**= control; **B** = 0.1; **C**= 1% and **D**= 2% concentrations; herb. = herbicide; conc. = concentration

Bars with similar letters are not significantly different at $p<0.05$ according to Duncan's multiple range test.

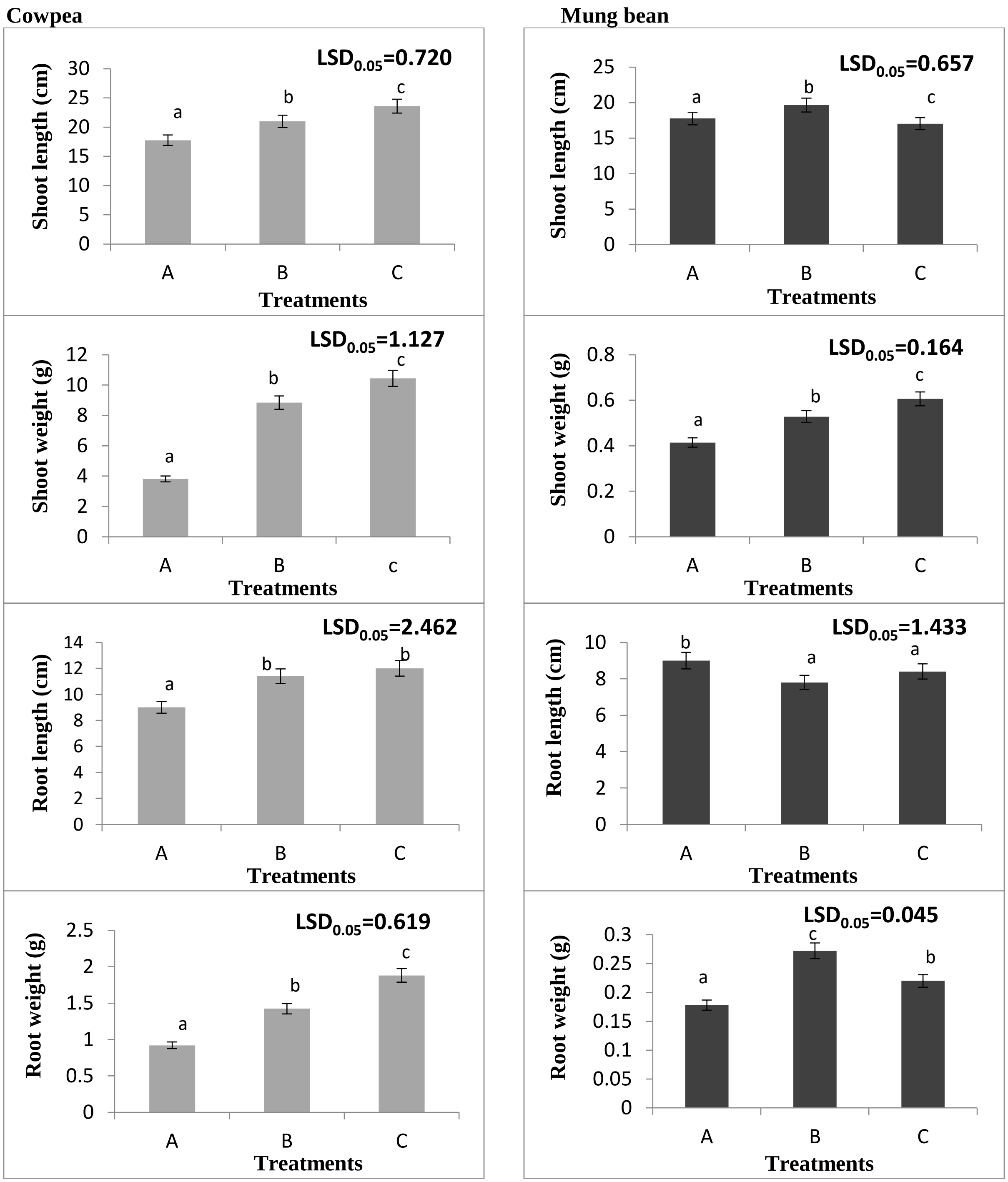

Figure 2 continued…

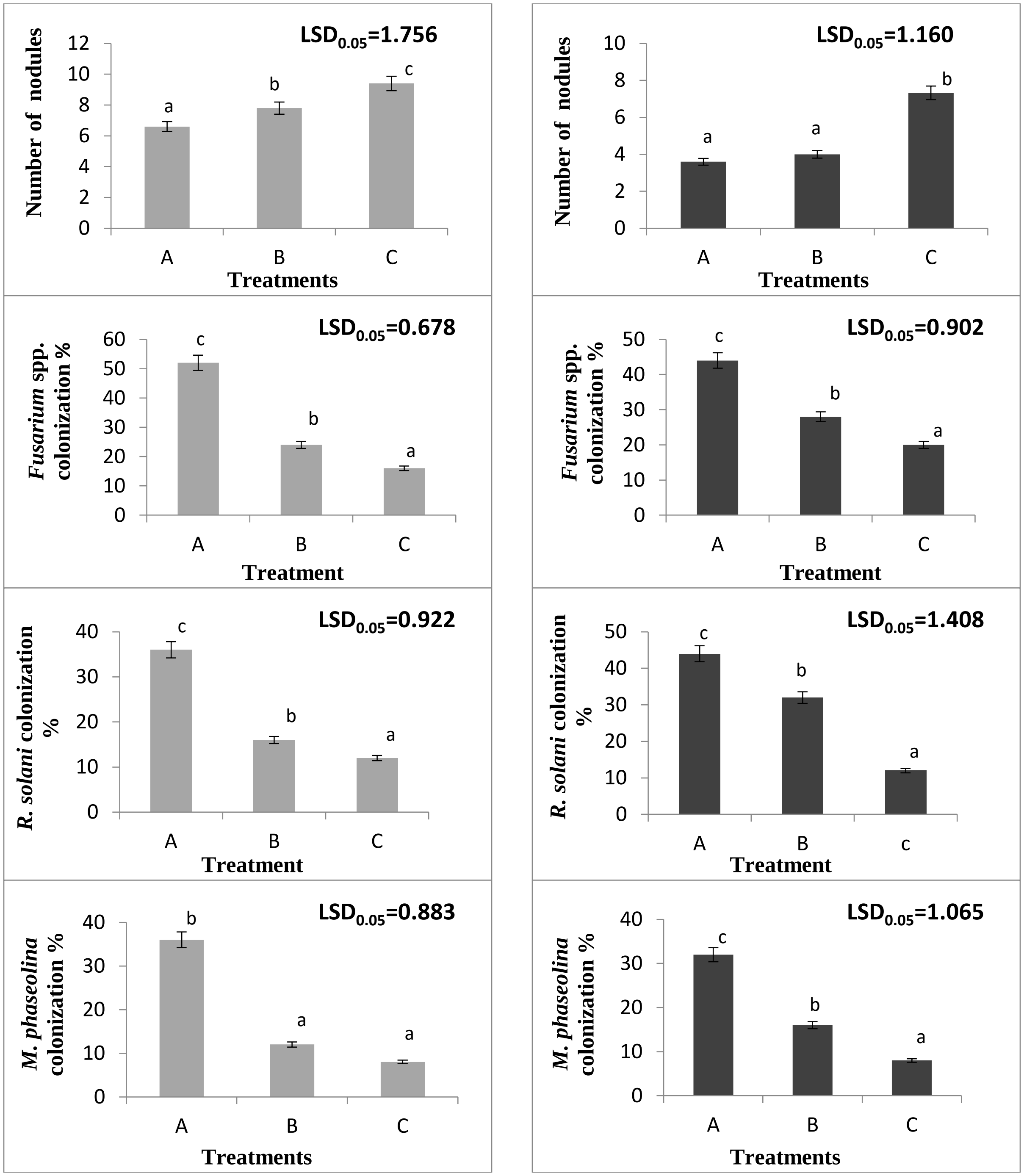

Fig. 2. Soil drenching with herbicides on growth parameters and in the control of root rot fungi on mung bean and cowpea plants under field condition.
A= control; **B**= 2% concentration of Bromacil; **C**= 2% concentration of Bromoxynil.
Bars with similar letters are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

It was interesting to note that the weeds were suppressed, when the soil was drenched with herbicides. The purpose of removing the weed from field is to reduce the competition because weeds emerge faster and rapidly than crop using the available resources like nutrients, moisture, sunlight and space during entire vegetative and early reproductive stages of chilli (Isik *et al*., 2009). The reduction in fruit yield of chilli was upto 60-70% which depends upon amount and persistence of weeds in standing crops **(**Patel *et al*., 2004). Application of herbicide in field depends largely on its effectiveness and economics. Sebiomo *et al.* (2011) reported that some microorganisms were able to degrade the herbicide, while some others were adversely affected depending on the application rates and the type of herbicide used.

The aim of the present studies was to investigate the potentiality of bromacil and bromoxynil in the control of root rot fungi which help in the growth of leguminous plants. It was concluded that these herbicides showed an antifungal activity which was found good for the development of viable mode of agriculture in farming system. Hence, more improvement can be easily done by using herbicides on a large scale by recording its effect on the growth and in the control of root infecting fungi.

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