

Comparison of Allelopathic Potential of Sri Lankan Improved Rice Varieties (*Oryza sativa* L.) using Radish (*Raphanus sativus* L.) as an Indicator Plant

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Abstract – Allelopathy is a sound alternative strategy to deal with weed management in paddy ecosystem in sustainable manner. Allelopathic influence imposes from chemicals which secrete from plants and called as allelochemicals. To avoid the hazardous and indiscriminate use of chemicals for weed control in paddy fields much concerns were deposited on proper assessment and manipulation of allelopathic properties of rice plants. There is a huge variation in allelopathic potential among rice varieties. Therefore, in this experiment relay seeding technique without soil (experiment 1) and soil as a medium (experiment 2) were conducted under laboratory condition to observe and screen the allelopathic potential of selected Sri Lankan improved rice varieties on seed germination, shoot and root length and dry matter production of radish (*Raphanus sativus*) as a phototoxic sensitive test crop. Most of the tested varieties of rice were performed inhibitions to studied characters of radish. In contrast some varieties act as stimulant rather than inhibition (At 306). In both experiments, the highest dry weight reductions were noticed with Ld 365 (53.8 and 56.9% respectively). Both the evaluation methods were positively correlated in determining percent reduction in all the measured parameters of radish. According to the results it can be concluded that the allelopathic potential of rice varieties varies in a very broad range and out of varieties tested Ld 365 showed highest allelopathic potential against radish growth.

Keywords – Allelopathy, Allelochemicals, Growth Inhibition, Radish.

I. INTRODUCTION

Rice is one of the most important crops in the world and also the staple food for peoples in Sri Lanka. Weeds are the crucial problem out of other circumstances which are adversely affected to the crop productivity of Sri Lankan rice cultivation. Due to heavy infestation of weed in paddy cultivation, rice yield and quality become deteriorate. Indiscriminate use of herbicides for weed control has caused for serious effect to the natural environment and living creatures including humans. Heavy use of herbicides is the root cause of number of inevitable primary and secondary hazards such as development of herbicide resistant weeds, shifts in weed population [1] enhancement of environmental pollution and human exposure to toxic materials [2], [3].

By concerning drawback of chemical weed control, in recent years, much concern has been raised about the positive aspects of environmental friendly and sustainable strategy of ecological weed management. Allelopathy is

defined as the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment [4]. Chemicals released from plants and imposing allelopathic influences are termed allelochemicals. Allelochemicals can be present in several parts of plants including roots, rhizomes, leaves, stems, pollen, seeds and flowers. Allelochemicals are released into the environment by root exudation, leaching from aboveground parts and volatilization and/or by decomposition of plant material [4], [5]. This phenomenon could be an alternative weed control method.

Since Dilday *et al.* [6] revealed that some rice accessions possess allelopathic activity in weed suppression, rice allelopathy has been received a great deal of attention and positive aspect as biological weed control method. A number of studies have been conducted to evaluate the allelopathic potential from rice germplasm and number of rice accessions having allelopathic potential have been determined in different places [2], [6], [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17], [18], [19]. Those experiments showed significant differences in allelopathic potential among rice varieties. Several allelochemicals were also extracted from different parts of rice plants and these chemicals are assumed to act in an additive or synergistic way rather than in an isolated way [20]. Some of the genes responsible for rice allelopathy have also been identified [21], [22]. However, so far a rice variety with higher allelopathic potential has not been developed. Although, it has been proved that the allelopathic potential of rice on weeds could be applied to reduce use of harmful synthetic herbicides, a proper study on allelopathic potential of commonly cultivating Sri Lankan rice varieties has not been conducted.

The major ways which plants emit allelochemicals to the environment are (1) plant residue itself or emission separated by microorganisms; (2) emission of water-soluble substances from plant root into the surrounding environments; (3) secretion of water soluble substances from plants above ground and come to the soil by rain, mist and/or dew; and (4) emission of gaseous substances from plant above ground to the atmosphere [23]. The substances found in plants are alkaloids, flavonoids, phenolics, terpenoids, lactones, tannins, cyanogenic glucosides, quinones, polyacetylenes, coumarins, sulfides and plant organic acids [24]. According to the Rimando *et al.* [25] and [26], most allelochemicals reported in rice were phenolic compounds and were found in leaf, straw, stem and rice soil rather than husk.

Allelopathic potential and resource competition is difficult to separate under field conditions. As a remedy for the above problem various laboratory screening techniques have been developed to measure allelopathy separating from the interference of resource competition. Several authors reviewed the importance and necessity of laboratory bioassays for initial allelopathic investigation in rice cultivars. Many researches have been showed that bioassays are the most convenient, effective and simplest way to estimate the allelopathic potential of rice as comparatively large number of rice cultivars can be examined in a limited time and space all year round [27]. Among several bioassays, sandwich method [28], agar medium selection [10], [29], plant box method [27] and relay seedling method [30] have been reported and tested for bioassays. Lettuce (*Lactuca sativa* L.), radish (*Raphanus sativus*) and cress (*Lepidium sativum* L.) were commonly used as indicator plants in those laboratory bioassays to screening rice allelopathy.

The present study was undertaken to examine the influence of commonly cultivating Sri Lankan improved rice varieties on seed germination and growth of radish and thereby evaluate the phytotoxicity of those rice varieties at their seedling stage.

II. MATERIALS AND METHODS

A. Plant materials

Forty commonly cultivating Sri Lankan improved rice cultivars (tab. 1) were used in this study. The selected rice varieties were collected from the four different rice research stations (Ambalanthota, Labuduwa, Batalegoda and Bobuwela) and the radish (*Raphanus sativus*) seeds were collected from the local market. The initial germination of the collected seeds was more than 80%.

B. Relay seeding method without soil

The relay seedling method described by [31] was followed in this study. Presoaked 20 rice seeds were placed on the petri dishes with Whatman No. 1 filter paper. Similar amount of distilled water was poured in each Petri dish to moist filter papers. Then the Petri dishes were kept at the laboratory conditions. The average room temperature ranged from 27 to 29°C. Fifteen clean and 12 hours water soaked seeds of radish were placed in each petri dish after 7 days of placement of rice seeds. In control treatment, 15 seeds of radish were placed in petri dish lined with soaked filter paper. The experiment was continued for another 7 days under the laboratory conditions. The treatments were replicated three times in CRD. Distilled water was added to each Petri dish every 2 days to maintain sufficient moisture for germination. The theme of this technique was that the rice seedlings released allelochemicals in the germination media and the growth of the neighboring test plant (radish) seedlings were affected due to allelochemicals.

C. Relay seeding method with soil

Experimental procedures were similar as described under relay seeding method without soil. However, instead to filter paper a soil mixture (top soil: sand: compost =

1:1:1) were used to ensure more stabilization of plants in the petridish.

D. Data collection

After 7 days of seed setting, number of germinated seeds, the root length and the shoot length of five randomly selected radish seedlings were measured. The sample seedlings were placed in paper bags and then kept in an electric oven at 65⁰ C for 48 h. The weights of oven dried specimens were recorded. The percent reduction in germination, root length, shoot length and dry matter accumulation was determined by using the following formula (Chung *et al.*, 2001).

$$\text{Inhibition percentage (\%)} = \frac{\text{control-rice cultivar}}{\text{control}} \times 100$$

Relationship between two methods in determining allelopathic potential of the rice varieties was also made using regression analysis. The collected data on different parameters of the indicator plant were statistically analyzed by using the SAS and the mean differences were adjudged using LSD.

III. RESULTS

Among the 40 rice varieties, the highest reduction in seed germination of radish under experiment conducted without a growing medium (experiment 1) was found with Ld 365 (55.8%) and closely followed by Ld 356 (49%) (tab. 1). There was no significant difference in between these two rice varieties in terms of inhibition of radish seed germination. Similar results were observed in relay seeding experiment conducted using soil as a growing medium (experiment 2) (tab. 1). All of the tested varieties inhibited the germination of radish seeds in both experiments.

The highest root length reduction in radish occurred with Ld 368 in both experiments (73.6 and 65% respectively) and the highest shoot length reduction was observed with Ld 356 (49.7%) in experiment 1 and with Ld 365 (58.5%) in experiment 2 (tab. 1). It was observed that few varieties stimulate the growth of shoot in both experiments. The highest dry weight reduction in radish was also noticed with rice variety Ld 356 (53.8 and 56.9% respectively) in both experiments.

The inhibition percentages according to pericarp colour were examined and brown rice showed significantly higher inhibition for all measured parameters in both experiments (tab. 3). Except dry matter accumulation highly significant positive relationship was noticed between the two experiments in measuring shoot length, root length, seed germination inhibition and dry matter accumulation of radish (fig. 1).

IV. DISCUSSION

The results of this study reveal that Sri Lankan improved rice varieties have significant effects on growth and development of indicator plant radish. Several plants such as radish, lettuce, cress, cucumber are phytotoxic sensitive plants widely used in allelopathic research [27].

Therefore, it is obvious that the wide variations in measured variables of radish were due to allelochemicals released from rice plants. Karim *et al.* [32] and Karim and Smail [33] have observed growth inhibition of lettuce seeds due to allelopathic effects of rice varieties. Further, similar kind of reduction in spinach due to allelopathic effects of rice varieties was noted by Kabir *et al.* [31]. Allelochemicals released from plants effect biological and biochemical processes of neighbouring plants and thereby it can be observed inhibition or stimulations of the growth and development of neighbouring plant. As an examples allelopathic compounds influence physiological processes such as cellular expansion, cell wall construction, phytohormonal balance, activity of specific enzymes, mineral uptake, photosynthesis, respiration, stomatal movements, protein synthesis *etc.* [4],[34],[35]. Except the effects of many physiological processes of neighbouring plants allelochemicals modify soil properties such as soil pH [36] and thereby indirect effects cause on plant growth and development.

In the present experiment it could be observed reduction in seed germination in both experiments (tab. 1). Previous research also reports delay in seed germination due to allelochemicals [36]. Findings of these researches revealed that several allelochemicals secrete from allelopathic species are structurally similar to plant hormones [37]. These allelopathic compounds can effect hormone induction during germination process or activity of specific enzymes such as amylases, proteinases *etc.* which are essential for seed germination [4]. In the present study except few varieties most of the varieties showed less than 15% germination inhibition (tab. 1) indicating that the allelopathic compounds release from tested rice varieties may not have much effect on inhibition of seed germination of radish. However, two Ld varieties *i.e.* Ld 365 and Ld 356 showed nearly 50% inhibition in seed germination. Therefore, allelochemicals release from these two varieties may have considerable effects on hormone activities necessary for seed germination.

The shoot and root length reduction of radish observed (tab. 1) in the present study may also due to the action of allelochemicals release from rice plants. Probably these allelopathic compounds may affect the nutrient uptake of radish plants and which ultimately interfered with the development of shoot length. However, few varieties showed stimulations of shoot length. Stimulations could be happened due to many reasons. First low concentrations of allelochemicals may cause stimulations to plant growth [4], second due to competition for light with neighbouring plants and finally allelochemicals may induce hormonal activities such as auxine necessary to increase shoot length. It could be observed reduced dry matter accumulation of radish in all varieties except At 306. Therefore, second and final reasons are more appropriate to discuss observed stimulations in shoot length of radish in the present study. It is obvious that most of the allelochemicals release from roots and root system of neighboring plants effect much, and finally those effects can be seen in all parts of the plant. In the present study except At 306 all other rice varieties showed reduced root

lengths (tab. 1). With some rice varieties stunted roots and browning of root tips and highly branched roots were observed. Similar kind of shoot and root length reductions and root discolorations were observed by many researches such as Karim and Smail [33] for *E. crusgalli*, Lie *et al.* [38] for *E. crusgalli*, Karim *et al.* [32] for lettuce and Kabir *et al.* [31] for spinach. Kim *et al.* [15] and Chung *et al.* [39] also observed similar detrimental effects of rice exudates on the root length of *E. crusgalli*. Further, Karim and Ismail [33] observed that rice varieties namely Manik and Makmuer caused more than 80% and 75% reduction of root length of *E. crusgalli*, respectively due to their allelopathic effect. Since allelopathic effect of rice varieties reduce shoot and root length of radish, the dry matter accumulation of radish was also reduced (tab. 1). Seedling weight is an important expression of size and vigor of the seedling which is an important feature for success in competition for sites, space, and nutrients [38], [40].

Rice varieties with higher allelopathic effect can be used as source of genes in rice breeding to develop high yielding and weed-suppressive rice varieties. Out of varieties tested Ld 365 showed the highest performance. The allelopathic studies conducted by Wathugala *et al.* [41] in field, green house and laboratory bioassays was also noted greatest inhibition of *E. crusgalli* by Ld 365. However, future work is needed to specify and verify the allelochemicals produced by these varieties.

There is a belief that varieties with coloured pericarp have high nutraceutical properties than white rice. Therefore, it could also be assumed that rice varieties with coloured pericarp may have higher allelopathic properties. Although not for coloured pericarp rice Jung *et al.*, [42] showed that rice residues of varieties with coloured hull have higher allelopathic potential on *E. crusgalli* than varieties with colourless hull. In this experiment it could be observed that varieties with coloured pericarp have significantly higher inhibitions of all measured parameters in both experiments (tab. 2).

A highly significant positive relationship was noticed between the two experiments in measuring shoot length, root length and germination percentage (fig. 1). For dry matter reduction comparatively less but positive correlation was observed (fig. 1). These observations indicate the appropriateness of using both the methods in investigating allelopathic potentiality of rice varieties.

Numerous numbers of researches has been conducted to evaluate allelopathic potential of rice germplasm in laboratory and green house screenings. Allelochemicals releasing to the environment highly differentiate qualitatively and quantitatively with plant growth and development stage and as well as highly inherent with plant origin. Therefore, further experiments under different climatic and management conditions and different developmental stages are important to understand allelopathic potential of rice varieties. Finally extraction and identification of particular chemicals would help to conclude allelopathic potential of various rice varieties. Although not for these rice cultivars several allelopathic chemicals such as p-hydroxybenzoic, ferulic, p-coumaric

syringic and salicylic acids from leaves and straws extracts, decomposing straw, and in rice soil have been identified in other countries [19].

In the present study radish was used as the test crop which is so sensitive to chemicals at low concentrations. Thus, some researchers argued that the species which are highly sensitive to phytotoxin sometimes overestimate the actual allelopathic activity of test plants [25]. Therefore, it is important to repeat this experiment with some common crops such as onion, sesame and bean as these plants did not react too sensitively against allelochemicals at low doses as lettuce, radish and cress did [21] and also with major paddy weed seeds such as *Echinochloa crus-galli*, *Monochoria vaginalis* etc. as they display well the allelopathic activities and the weed suppression potential of the screened rice cultivars.

V. CONCLUSION

In the present study significant differences ($p \leq 0.05$) among different rice cultivars in terms of inhibition of radish seed germination and seedling growth were observed. On the basis of measured variables Ld365 had highest allelopathic potential than other tested rice cultivars. Therefore, it can be concluded that Ld365 may have potentials to produce chemicals that are reduced weed germination and growth at seedling stage. However, allelopathic effect derives due to the allelochemicals and their bio active concentration. With the change of environmental, management and edapic factors these results may be deviated. Therefore, further recommendations of these results must need experiments on different environmental and management conditions as well as allelochemical extraction and identification.

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Table 1: Percent reduction in germination (GI%), root length (RLI%), shoot length (SLI%) and dry matter production (DWI%) of radish due to allelopathic effect of different rice varieties under relay seedling without soil and soil as a medium experiments.

Rice variety	PC	Experiment 1- Relay seeding without soil				Experiment 2- Relay seeding soil as a medium			
		GI%	SLI %	RLI %	DWI%	GI%	SLI%	RLI %	DWI%
Bw 400	B	15.0	22.1	26.4	25.8	16.1	14.8	25.3	26.1
Bw 452	B	35.4	18.6	33.5	6.7	34.0	18.9	32.8	21.6
Bw 302	W	6.0	-24.1	35.8	22.2	4.8	-27.2	29.3	29.7
Bw 267-3	W	13.8	-15.7	27.4	35.4	11.6	-17.4	20.6	16.7
Bw 451	W	11.6	24.0	12.8	29.6	11.6	17.9	33.6	22.9
Bw 361	B	25.2	24.7	28.4	19.1	18.4	21.8	19.9	22.5
Bw 363	W	13.4	18.7	16.9	31.2	12.2	31.1	26.6	38.9
Bw 367	W	15.0	33.5	17.6	16.5	13.3	15.9	25.6	18.8
Bw 364	B	15.0	-3.5	36.4	35.1	18.4	-22.0	36.4	13.4
Ld 365	B	55.8	42.2	65.6	53.8	59.1	58.5	64.6	56.9
Ld 356	B	49.0	49.7	36.7	16.3	45.6	47.3	27.4	19.6
Bw 272-6b	B	11.1	26.6	27.1	24.3	13.3	14.4	24.6	20.6
Ld 368	B	25.2	18.4	73.6	20.2	37.1	23.5	65.0	21.9
Ld 408	B	15.0	47.3	60.7	20.5	32.8	44.7	50.0	17.7
Bw 453	W	6.1	4.0	49.0	34.0	6.7	5.8	46.4	28.6
Ld 355	W	15.0	47.5	48.1	26.7	53.4	35.3	37.9	19.7
Bg 354	W	6.3	21.4	43.2	27.1	6.0	27.8	44.9	11.0
Bw 351	W	16.1	3.9	14.1	32.1	16.1	2.3	12.2	23.6
At 307	W	6.3	38.7	23.8	11.8	6.7	26.1	26.6	16.4
Bg 407	W	17.0	-28.3	30.0	15.1	6.7	-23.4	30.4	25.9
At 353	W	12.6	22.1	16.7	27.8	12.2	20.8	13.4	22.1
At 402	B	11.1	26.4	30.1	37.3	13.3	26.8	33.0	21.7
At 362	B	12.0	42.9	61.9	19.9	11.6	31.4	59.1	23.3
H-4	W	14.5	5.7	24.8	15.8	13.3	4.1	20.9	19.2
Bg 406	B	13.8	40.9	35.3	22.6	13.8	31.7	32.2	26.2
At 308	W	13.7	26.3	19.8	26.5	13.3	16.9	24.9	27.2
Bg 305	W	10.0	22.2	41.5	19.4	10.5	18.4	31.9	15.8
Bg 3-5	W	6.7	14.2	24.8	3.3	6.7	14.3	28.8	7.4
Bg 304	W	6.7	32.2	21.5	21.6	6.7	23.3	18.8	24.1
Bg 38	W	10.0	16.4	21.5	6.9	12.2	19.1	16.2	7.1
Bg 403	W	6.7	5.8	31.3	24.3	6.7	2.8	23.1	17.3
At 303	B	10.0	30.9	34.2	29.4	14.4	25.3	30.8	15.6

Bg 360	W	13.3	12.8	39.6	7.7	13.3	12.1	28.6	7.2
Bg 454	W	13.0	23.1	22.3	21.6	13.0	25.3	28.8	22.1
Bg 407 -H	W	6.7	21.1	10.7	15.5	6.7	21.2	16.0	21.6
Bg 359	W	6.0	15.0	25.1	10.2	4.5	11.8	35.6	26.6
Bg 358	W	5.8	17.1	44.0	14.2	2.2	19.8	36.1	24.6
At 306	W	12.1	-16.8	-11.4	-6.0	12.1	-26.6	-10.2	-5.3
At 401	B	8.2	27.3	21.9	44.9	10.5	22.3	18.0	30.6
At 405	W	7.0	32.4	32.8	13.4	6.1	24.7	29.7	28.4
CV			4.6	5.1	7.2	9.6	4.5	5.2	7.2
LSD			0.5	0.5	0.6	0.5	0.5	0.5	0.6

PC – Pericarp colour, ‘B’ and ‘W’-brown and white pericarps respectively.

Table 2: Percent reduction in germination (GI%), root length (RLI%), shoot length (SLI%) and dry matter production (DWI%) of radish considering pericarp colour of different rice varieties under relay seeding without soil and soil as a medium experiments.

Pericarp colour	Experiment 1- Relay seeding without soil				Experiment 2- Relay seeding soil as a medium			
	GI%	SLI %	RLI %	DWI%	GI%	SLI%	RL I%	DWI%
Brown	21.5	29.6	40.8	28.3	24.2	25.7	37.1	24.1
White	10.4	14.4	26.3	18.9	11.2	12.1	26.2	19.8
CV	8.4	6.0	3.3	5.4	4.7	5.6	1.2	3.6
LSD	3.0	3.0	2.5	2.9	1.9	2.4	0.9	1.7

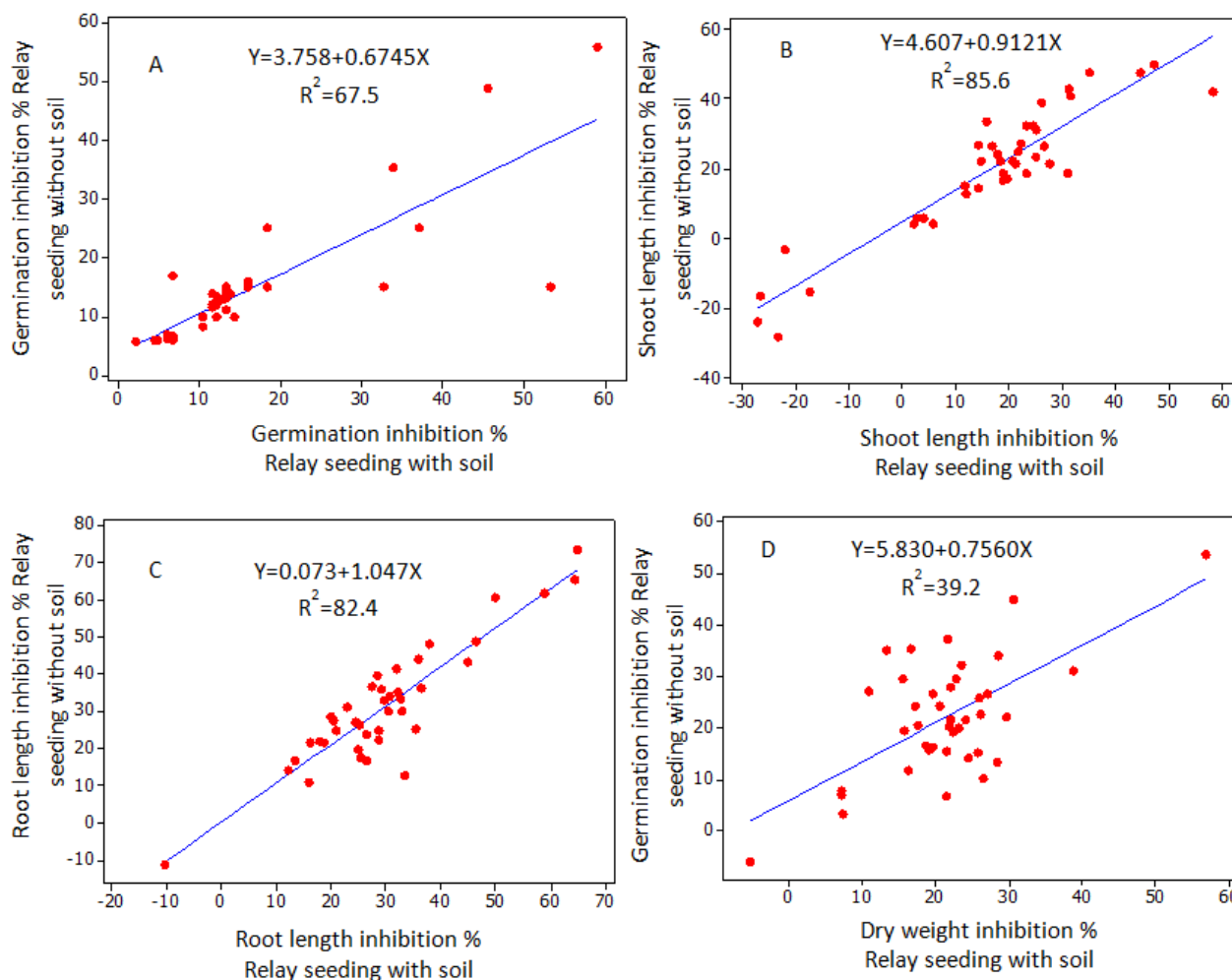


Fig.1. Correlation between two methods in measuring (A) Seed germination (B) shoot length, (C) root length and (D) dry matter production of radish growth