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Soil fertility improvement by addition of bio-fertilizer *Azolla pinnata* R Br and its utilisation in a eco-friendly way

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Abstract
The alarming rate of contaminations of fertilizers in soil has become an important issue worldwide due of their undesirable effects on environment, human health and serious threat to food safety. Excessive addition of synthetic fertilizer leads to chemical pollution in soil. Chemical pollutants can easily enter the food chain if contaminated soils are used for the production of food crops. So, reducing the effects of heavy metal on food crop, an attempts were taken to use of *Azolla pinnata* R. Br. as a bio-fertilizer. The novelty of this work is the replacement of synthetic fertiliser by utilization of *Azolla pinnata* R. Br. as biofertiliser in an environment friendly way. This research address the research gap by analyzing the elemental content present in *Azolla pinnata* R. Br. In the present study it was found that the *Azolla pinnata* R. Br. contains different useful elements which are major and minor element for plant nutrient. In the present study, dry *Azolla pinnata* R. Br. powder was added to the garden soil and elemental contents of both initial and final soil were measured and effect of these nutrients on growth of *Vigna radiate* L. were analyzed. It was observed that the soil treated with *Azolla pinnata* R. Br. biomass contained more nutrient than the normal garden soil. This biomass treated soil was also utilized for growing *Vigna radiate* L. seedling. It was also found that the *Vigna radiate* L. seedlings was grown better in biomass treated soil than the normal garden soil. So *Azolla pinnata* R.Br. can be recommended as the organic fertilizer which can fulfil the nutrient level of soil and enhance the soil fertility.

Keywords: *Azolla pinnata* R. Br., soil, Elemental analysis, bio-fertilizer

1. Introduction
The alarming contaminations of fertilizers in atmosphere, lithosphere, hydrosphere and biosphere have become an important issue worldwide because of their adverse effects on human health and serious threat to food safety. Chemical pollutants can easily enter the food chain if contaminated soils are used for the production of food crops. Some of heavy metals (iron, copper and zinc) are essential for plants. The availability of heavy metals in medium varies, and metals such as copper, zinc, iron, manganese, molybdenum, nickel and cobalt are essential micronutrients, whose uptake in excess to the plant requirements result in toxic effects. They are also called as trace elements due to their presence in trace or in ultra-trace quantities in the environmental matrices.
In this regard, recent efforts have been channelized more towards the production of 'nutrient rich high-quality food' in sustainable comportment to ensure bio-safety. The innovative view of farm production attracts the growing demand of biological based organic fertilizers exclusive of alternative to agro-chemicals. Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with indiscriminate use of agrochemicals.

2. Experimental section
In this study, soil samples were collected from a garden of Centurion University of Technology and Management, BBSR, Odisha, at depths of 18 cm using soil auger.
The samples were collected in a polyethylene bags and properly labelled. Then the collected samples were taken to the laboratory and treatment was done for the preservation of soil and further analysis has been done as per standard procedure[1]. The collected samples were air dried in sun light for about twenty-four hour. Then the samples were dried in an oven at 105˚C till complete dehydration. Then the sample was ground in a mortar pestle then passed through 0.5 mm nylon mesh sieve. These soil samples were again packed with the complete labelling and preserved for further analysis. *Azolla pinnata* R.Br. was collected from Azolla tank of CUTM garden, Bhubaneswar, Odisha. The elemental content of both soil and azolla treated soil were analyzed using standard methods.
Uniform sized seeds of *Vigna radiate* L. were selected and surface stained with 0.1% mercuric chloride ($HgCl_2$) for about five minutes for sterilization and then was washed several times with tap water followed by distilled water. The surface sterilized (0.1% $HgCl_2$) seeds were germinated in sterilized Petri plates over Whatman filter paper.10ml of distilled water (for control) or solutions of *Azolla pinnata* R.Br. containing specific concentration of *Azolla pinnata* R.Br. (200mg, 400mg, 600mg, 800mg and 1000mg) was poured into each petri-plates. Seeds were allowed to germinate at room temperature (28 ± 2ºC) in darkness for five days. The number of seed germinated in each treatment was counted and radical length of roots was also measured.
Germination percentage was calculated by using the formula:

$$\text{Germination \%} = \frac{\text{Number of seed germinated}}{\text{Total number of seed shown}} \times 100$$

According to Chou et. al (1978), The percentage of phytotoxicity of the metal was calculated by the formulae:

$$\text{Phytotoxicity (\%)} = \frac{\text{Radicle length of control} - \text{Radicle length of test}}{\text{Radicle length of control}} \times 100$$

Seedling vigour indices were calculated by using the formulae proposed by Baki and Anderson (1973).
Seedling Vigour index = Germination percentage × Radicle length
According to Turner and Marshal, 1972, the tolerance indices of the seedlings were calculated as:

$$\text{Tolerance index} = \frac{\text{Radicle length of seed in test}}{\text{Radicle length of control}} \times 100$$

Growth parameter study
The growth parameters like root length, shoot length, fresh matter, dry matter, etc. of 15days and *Vigna radiate* L. seedlings were used for study. Different *Azolla pinnata* R.Br. concentrations (200mg, 400mg, 600mg, 800mg and 1000mg) and one untreated soil were used during growth parameter study.

Root length and Shoot length
For study of root and shoot length of 15 days old plants, the roots and shoots were first detached from each other. Individual length of root and shoot was measured in centimetre.
All the experiments were done in triplicates and the data presented in the figures are the means of three independent experiments. The data were analysed statistically and standard errors of mean (SEM) were given wherever required.

3. Results and Discussion
In this study, different physico-chemical parameters and growth parameter of *Vigna radiate* L. Using garden soil and garden soil treated with Azolla. Parameter like elements/compound contents were analysed and both the results were compared with their growth parameters (Table-1).
There are several elements and compounds were found to be present in Azolla which enhanced the nutrient level of garden soil. After addition of Azolla, concentration of different valuable elements like P2O5, SO3, Cl, K2O, CaO, Br, CuO, SrO and Fe2O3 were found to be increased which enhanced the soil nutrient level. It was reported that Nitrogen, phosphorus, and potassium fertilization to achieve expected yield and improve yield components of mung bean [2, 3], similarly various studies like growth yield of mung bean in different level of pottacium [4], the effect of phosphorous on growth of mung bean were reported [5]. Iron helps the plant move oxygen throughout the roots, leaves, and other parts of the plant, producing the green colour that lets you know your plant is healthy. Many plants also rely on iron to complete the enzyme functions that keep the plant thriving. Copper activates some enzymes in plants which are involved in lignin synthesis and it is essential in several enzyme systems. It is also required in the process of photosynthesis, is essential in plant respiration and assists in plant metabolism of carbohydrates and proteins. Similarly, another report was found on increasing levels of Phosphorus and Sulphur enhanced the growth, Plant height, yield attributes like Number of nodules/plant, Dry weight of nodules, Number of pods/plant, Number of grains/pod and grain yield [6].
This Azolla pinnata used as organic fertiliser and tested the seedling pattern and growth parameter of Vigna radiate L. This mixture means Azolla treated with soil showed variable impacts on germination and seedling growth of Experimental plant. The seeds were germinated in laboratory in different concentrations of fertiliser 00 mg/l to 1000 mg/l. Simultaneously another set of seeds were germinated at garden with varying concentration 00 g/kg to 50 g/kg of Azolla in soil. From this study it was observed that the percentage of germination increased by increasing the concentration of Azolla pinnata. The percentage of germination of seeds was increased from 36.5% to 88.25% in treatments of 00 mg/kg to 1000 mg/kg of Azolla respectively. Similarly, the root and shoot lengths of germinated seedlings in pot culture were found to be increased with increasing concentration of Azolla pinnata. When the seeds were grown in soil mixed with Azolla for 15 days, the root and shoot length was also found to be increased with increasing concentration from control to 1000gm/kg of Azolla mixed with soil. Because of the increasing micro and macro nutrient due to the mixture of Azolla pinnata with soil, become the main reason for the growth of shoot and root. Such evidence was also found that an experiment was conducted on effect of Phosphorous on Growth and yield of mungbean which showed a good result on development of the experimented plant [7]. In a study it was reported that the application of egg shell waste enhance the soil fertility [8]. Similarly, a report suggested that Azolla pinnata using as a biofertilizer responsible for producing healthy tomatoes [9]. The presence of some metals like copper, zinc, iron, manganese, molybdenum and nickel are essential micronutrient for plant growth [10]. Similarly, small amount of different metals enhance the plant growth like nickel in *Macrotyloma uniflorum* [11]. From the above observations on the germination study of *Vigna radiata*, it was clearly earmarked that the germination% increased & inhibition% decreased and radical length was increase with increase of concentration of *Azolla pinnata* R.Br. Seedling vigour index and tolerance index were increased with increase of concentration of *Azolla pinnata* R.Br while the phytotoxicity was increased with increase of *Azolla pinnata* R.Br concentration.

Table 1: Different elements/compounds present in garden soil, fruit waste and garden soil mixed with Azolla.

Elements/ Compounds	Unit	Garden Soil	Azolla	Garden soil + Azolla
Al_2O_3	%	16.850±0.005	00	16.042 ± 0.082
SiO_2	%	67.203±0.003	8.141±0.0073	63.21±0.065
P_2O_5	%	1.026±0.004	4.919±0.0057	1.508±0.005
SO_3	%	00	3.720±0.0058	0.149±0.003
Cl	%	00	20.49±0.0065	0.196±0.003

K_2O	%	2.261±0.004	17.410±0.0057	3.071±0.004
CaO	%	4.242 ± 0.064	37.624±0.0063	6.980±0.004
TiO_2	%	0.535±0.003	0.622±0.0043	0.48±0.055
MnO	%	0.134±0.003	1.461±0.0052	1.194±0.002
ZrO_2	%	0.124±0.003	0.006±0.001	0.108±0.002
Fe_2O_3	%	0.124±0.003	5.159±0.0067	0.148±0.002
ZnO	%	193.9±0.82	0.3302±0.2199	150.3±0.63
Cr_2O_3	Ppm	2.271±0.089	00	2.081±0.024
NiO	Ppm	553.0±1.324	202.5±1.82	371.6±0.28
V_2O_5	Ppm	662.3±2.426	571.1±2.82	581.0±1.125
Ga_2O_3	Ppm	74.6±0.68	00	51.5±0.08
Rb_2O	Ppm	147.8±1.42	00	140.4±1.23
As_2O_3	Ppm	102.2±1.28	21.2±0.08	61.2±0.05
SrO	Ppm	32.9±0.86	323.8±1.46	158.5±1.59
Y_2O_3	Ppm	13.4±0.08	00	11.3±0.61
Nb_2O_5	Ppm	136.8±1.24	00	73.5±0.53
SnO_2	Ppm	132.6±0.98	00	103.7±1.29
Eu_2O_3	Ppm	37.6±0.82	00	35.6±1.49
Br	Ppm	00	386.2±1.22	0.285±0.002
ThO_2	Ppm	48.9±0.64	00	43.5±0.58
Re	Ppm	35.4±0.62	00	17.6±0.63
CuO	Ppm	336.8±1.286	479.3±2.08	407.4±1.65

Values of four replicates ± SEM

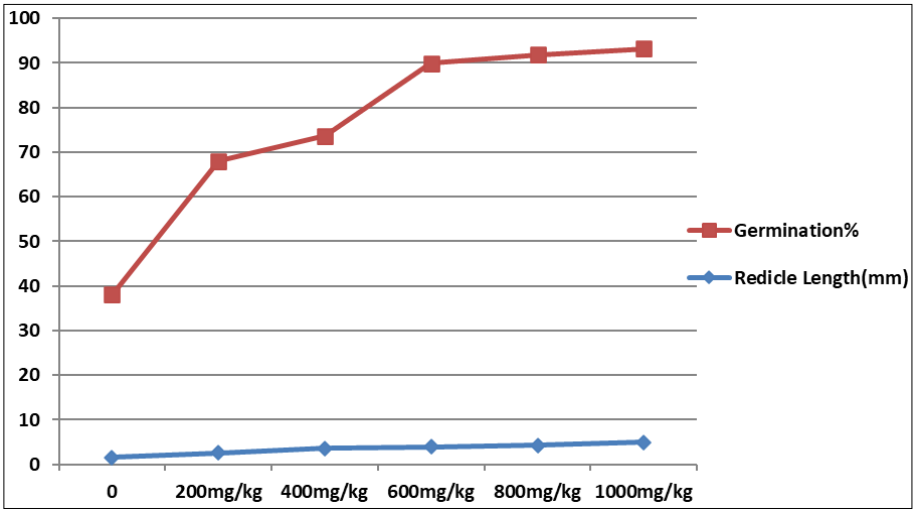

Fig 1: Impacts of different concentration of *Azolla pinnata* R.Br. on seed germination.

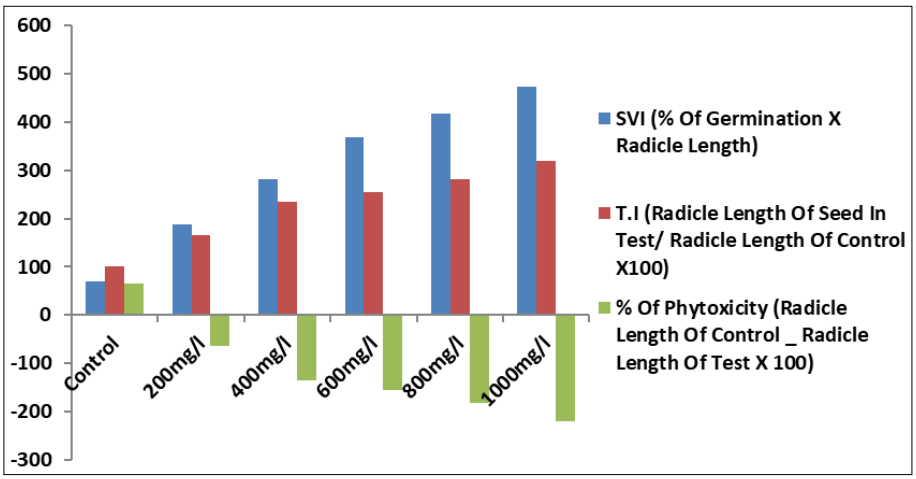

Fig 2: Impact of *Azolla pinnata* R.Br. on seedling of *Vigna radiate* L. vigour indices, tolerance indices and percentage of toxicity

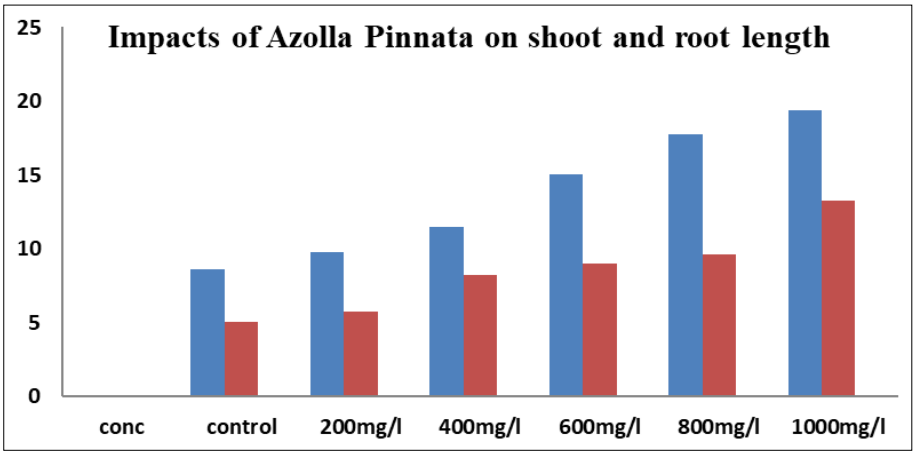

Fig 3: Impact on the growth of *Vigna radiate* L. seedlings after 15 days of treatment of *Azolla pinnata* R.Br.

4. Conclusion

Azolla can be used as plant fertilizer due to presence different valuable macro and micro nutrient for plants. In the present study, it was found that the mungbean seedlings were grown better on Azolla treated soil than the seedlings grown in control. So, this research can say that Azolla can fulfil the mineral requirement for the plants growth. Overall, it is excellent organic manure for *Vigna radiate* L. (mung bean) plants. It can be recommended for utilization at crop field for better growth and development of plant without causing any harm to the plant.

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