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Microbial bio inoculants coupled with weed extracts as biostimulants for enhancing yield of isabgol *(Plantago ovataForsk)*

Abdul Mazeed[1], Nihal Dwivedi, Dipender Kumar[2], Priyanka Suryavanshi[1]
1. Division of Crop Production and Protection, CSIR- Central Institute of Medicinal and Aromatic Plants, Lucknow-226 015, India
2. Division of Crop Production and Protection, CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre, Pantnagar, Uttarakhand-11 263149, India
Corresponding author's email: priyanka@cimap.res.in

ABSTRACT

Isabgol is cultivated for its export value as its seed and husk are used for medical purposes. Being natural in nature with no side effects, its demand in USA and other West European countries as a household medicine is increasing. To enhance the growth and yield of Isabgol, an experiment was conducted to study the effects of weed leaf extracts as bio-stimulants along with the bio-fertilizers.Four commercial products are combined with microorganisms (Pusa zinc solubilizing bio-fertilizer, PusaAzotobacter liquid bio-fertilizer, Pusa PSB liquid bio-fertilizer, Pusa Potash solubilizing liquid bio-fertilizer) along with extracts of four weeds [Cyperusrotundus L., Amaranthusviridis L., Echinochloacolona (L.) Link, DigeraarvensisForsk.]. The treated plants demonstrated stimulatory reactions in growth and physiology, which resulted in higher Isabgol seed and husk production compared to the control. The use of these bio-stimulants and bio-fertilizers together had a synergistic impact. Among all the treatments Pusa PSB liquid bio-fertilizer along with Amaranthusviridisweed leaf extract gave significantly highest plant canopy (67.33 cm) and dry matter (42.33 g/pot), PusaAzotobacter liquid bio-fertilizer along with Amaranthusviridisweed leaf extract gave significantly highest number of spikes/plant (114), Pusa PSB liquid bio-fertilizer along with Cyperusrotundusweed leaf extract gave significantly highest fresh biomass (101.33 g/pot) PusaAzotobacter liquid bio-fertilizer along with Amaranthusviridisweed leaf extract gave significantly highest husk yield (8.33 g/pot) while Pusa PSB liquid bio-fertilizer along with Cyperusrotundusweed leaf extract gave significantly highest seed yield (21.33 g/pot). All of these findings greatly indicate the potential of these weed leaf extracts when used with certain bio-fertilizers as it is an environment friendly approach to enhancing growth and yield of Isabgol crop.

Keywords: Biostimulants, weed extracts, microbial inoculants, isabgol, yield

INTRODUCTION

For many centuries South Asia has used Isabgol as an Ayurvedic herb in health care and now it is being used all over the world for its great medicinal properties. India holds a dominant stance in the production and export of Isabgol all over the world. Among all the other medicinal crops, Isabgol ranks first in earning the highest foreign exchange for India (Rs. 3 Crores annually). India contributes near about 30% of the total Isabgol production occupying 2.65 lakh ha area. Although India is the largest producer of Isabgol in the world, its productivity is still not enough to meet the global demand. Isabgol husk is obtained from ripe seeds of Isabgol. A variety of formulation properties of Isabgol seeds and husks can be found in pharmacology as laxatives, used to treat disorders of the gastro-intestinal and reproductive tracts, constipation, dysentery, and ulcers of the duodenum and piles, in addition to inflammation of these mucous membranes and lowering blood cholesterol level. Isabgol is generally cultivated in Madhya Pradesh, Rajasthan, Gujarat, and Haryana. In terms of area and production of this crop, Rajasthan ranks first in the country (67%). Water requirement of this crop is very less as compared to the other crops making it very suitable for these areas.

Weeds accumulate a lot of nutrients from the cropping soil and thereby can play an important role as a potential bio-stimulant. The higher nutrient concentrations in weeds' respective biomass could also act as a biostimulant due to the fact that they accumulate nutrients from cropped soil. Their tissue tends to accumulate macronutrients and micronutrients at a quicker rate than other crops as reported by Rao and Matsumoto (2017). It has been reported that weed leaves extracts are able to increase chlorophyll content, pod number plant-1, and yield of beans (Mkindi *et al.,* 2000).Hence an experiment was conducted to study the potential of these weed leaf extracts as bio-stimulants and their combination with bio-fertilizers in enhancing the growth and yield of Isabgol.

MATERIALS AND METHODS

Experimental site/location

The experiment took place in the CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP) research farm, which is located at 26°5' N latitude and 80°5' E longitude, with an elevation of roughly 120 m above mean sea level in Lucknow, Uttar Pradesh. The annual average rainfall at the location was approximately 1000 mm. The pot experiment was carried out from October 2020 to April 2021, and the soil utilised was loamy sand with a pH of 8.05 and organic carbon content of 3.21 g / kg soil, Nitrogen 202.1 kg / ha soil, Phosphorous 52.06 kg / ha soil, and Potassium 152.24 kg / ha soil, respectively.

Crop cultivation

Quality seed material of *Plantagoovatawas* obtained from CSIR-CIMAP's gene bank, Lucknow. For the cultivation of the Isabgol crop, standard agronomic practices were used. All of the pots received the needed dose of bio-fertilizer via microbial cultures, with the quantity of those microbial cultures varying according to treatment. Weed leaf extracts were sprayed after seeding for the required time. All additional cultural activities, including as weeding, irrigation, and foliar spray, were carried out in accordance with the crop's needs during its growth phase.

Treatment combination and experimental design

Design used for the pot experiment was completely randomized block design (CRBD) with seventeen treatments and three replication along with control. Four commercial products with microorganisms (Pusa zinc solubilizing bio-fertilizer, PusaAzotobacter liquid bio-fertilizer, Pusa PSB liquid bio-fertilizer, and Potassium solublizing bacteria) were combined with leaf extracts from four weeds (*Cyperusrotundus* L., *Amaranthusviridis* L., *Echinochloacolona* (L.), *Digeraarvensis*Forsk.). The treatments thus formed were Pusa zinc solubilizing bio-fertilizer + *Cyperusrotundus* leaf extract; Pusa zinc solubilizing bio-fertilizer + *Amaranthusviridisleaf* extract; Pusa zinc solubilizing bio-fertilizer + *Echinochloacolonaleaf* extract; Pusa zinc solubilizing bio-fertilizer + *Digeraarvensisleaf* extract; Pusaazotobacter liquid bio-fertilizer + *cyperusrotundusleaf* extract; Pusaazotobacter liquid bio-fertilizer + Amaranthusviridisleaf extract; Pusaazotobacter liquid bio-fertilizer + *Echinochloacolonaleaf* extract; Pusaazotobacter liquid bio-fertilizer + *Digeraarvensisleaf* extract; Pusa phosphate solubilizing bacteria (PSB) liquid bio-fertilizer + *Cyperusrotundusleaf* extract; Pusa PSB liquid bio-fertilizer + *Amaranthusviridisleaf* extract; Pusa PSB liquid bio-fertilizer + *Echinochloacolonaleaf* extract; Pusa PSB liquid bio-fertilizer + *Digeraarvensisleaf* extract; Potash solubilizing liquid bio-fertilizer + *Cyperusrotundusleaf* extract; Potash solubilizing liquid bio-fertilizer + *Amaranthusviridisleaf* extract; Potash solubilizing liquid bio-fertilizer+ *Echinochloacolonaleaf* extract; Potash solubilizing liquid bio-fertilizer+ *Digeraarvensisleaf* extract and control. To maintain the same moisture content, control plants were sprayed with distilled water.

The stock of bio-fertilizers was used to prepare fresh solutions for use. A 0.2% solution was prepared by adding tap water to the stock. Seed treatments and soil applications of liquid bio-fertilizers were administered during the seeding process. A foliar application of each weed leaf extract was made twice consecutively at intervals of 60 days and 75 days after sowing. In order to apply weed leaves extracts to the leaves, a 5 ml graduated atomizer or sprayer was utilised.

Preparation of weed leaf extracts

Weed leaves were collected from the research farm of the CSIR-CIMAP, Lucknow. For two weeks, we air-dried one kg of each weed's leaves under shade. We then ground it using a pestle and mortar. We prepared the 1% stock extract by soaking 10 g leaf powder in 1 liter distilled water and shaking it on a shaker for 24 hours at 25°C. Purifying the extract involved filtering it twice through (Whatman No. 1) filter paper. The extracts were utilised within five hours after being chopped and extracted (Mahdavikia and Saharkhiz 2015).Extracts of weed leaf nutrients and their chemical composition were analyzed (Table 1).

Crop harvesting and observations related to plant growth

Plant height (cm), plant canopy (cm), No. of tillers / plant, Number of spikes / plant, Fresh biomass (g / pot), Dry matter (g / pot), seed / plant, husk / plant, test weight were measured from each plant of every pot.

Plant growth observations

Plant height, plant canopy, number of tillers / plant were measured from the pots during the growth stage of the plants while plant height, plant canopy, number of spikes / plant, fresh biomass (g / pot), dry matter (g / pot) were measured during the harvest stage. After the harvest seed yield and husk yield were measured in gram / pot and weight of 1000 seed was also recorded.

Statistical analysis

Shapiro–Wilk test of the data sets were conducted to check the normality distribution at a 5% level. One way analysis of variance was applied to study the influence of various treatment on growth and yield of Isabgol. The data recorded was analysed using Post hoc Duncan's test to find out the significant variations between means of different treatments at $p<0.005$. SPSS 25 was the statistical software used.

Table 1.
Influence of bio-fertilizers and weed leaf extracts on Isabgol crop growth parameters at growth stage

Treatment	Plant height(cm)	Plant canopy(cm)	Number of tillers/plant
Control (sprayed with distilled water)	18.67	36.667	7.333
Pusa zinc solublizingbio-fertilizer + *Cyperusrotundus*leaf extract	25.33	52.333	12.333
Pusa zinc solublizingbio-fertilizer + *Amaranthusviridis*leaf extract	21.67	40.667	16.000
Pusa zinc solublizingbio-fertilizer + *Echinochloacolona*leaf extract	21.67	39.667	9.000
Pusa zinc solublizingbio-fertilizer +*Digeraarvensis*leaf extract	20.00	33.333	9.667
PusaAzotobacter liquid bio-fertilizer + *Cyperusrotundus*leaf extract	25.00	42.667	12.333
PusaAzotobacter liquid bio-fertilizer + *Amaranthusviridis*leaf extract	26.67	42.333	13.000
PusaAzotobacter liquid bio-fertilizer + *Echinochloacolona*leaf extract	22.33	37.333	16.667
PusaAzotobacter liquid bio-fertilizer + *Digeraarvensis*leaf extract	21.67	29.000	19.333
Pusa PSB liquid bio-fertilizer + *Cyperusrotundus*leaf extract	25.33	46.000	17.667
Pusa PSB liquid bio-fertilizer + *Amaranthusviridis*leaf extract	24.33	47.333	19.333
Pusa PSB liquid bio-fertilizer + *Echinochloacolona*leaf extract	21.00	47.333	10.000
Pusa PSB liquid bio-fertilizer +*Digeraarvensis*leaf extract	24.33	45.000	14.333
Potash solublizing liquid bio-fertilizer+ *Cyperusrotundus*leaf extract	22.67	43.667	19.500
Potash solublizing liquid bio-fertilizer+ *Amaranthusviridis*leaf extract	19.67	35.667	13.667
Potash solublizing liquid bio-fertilizer+ *Echinochloacolona*leaf extract	22.33	42.333	14.667
Potash solublizing liquid bio-fertilizer+*Digeraarvensis*leaf extract	22.67	40.000	15.000

Note: No significant difference between the treatments when same letter follows the mean in the same column (DMRT)

Table 2.
Influence of bio-fertilizers and weed leaf extracts (WLE) on Isabgol crop growth parameters at harvest stage

Treatment	Plant height(cm)	Plant canopy(cm)	No. of spikes plant^{-1}	Fresh biomass(g pot^{-1})	Dry matter(g pot^{-1})
Control (sprayed with distilled water)	32.000	39^{ab}	93^{ab}	56.33^{abcde}	34.33^{a}
Pusa zinc solublizingbio-fertilizer + *Cyperusrotundus*leaf extract	36.333	49^{abcd}	108.33^{efg}	74^{cdef}	40de
Pusa zinc solublizingbio-fertilizer + *Amaranthusviridis*leaf extract	34.000	47^{abcd}	102.33^{bcde}	48^{abc}	38.67^{bcde}
Pusa zinc solublizingbio-fertilizer + *Echinochloacolona*leaf extract	36.667	65^{cd}	95.33^{abc}	73^{cdef}	37.33^{abcd}
Pusa zinc solublizingbio-fertilizer +*Digeraarvensis*leaf extract	34.333	45.67^{abc}	103.67^{cdef}	64.33^{bcdef}	40.33^{de}
PusaAzotobacter liquid bio-fertilizer + *Cyperusrotundus*leaf extract	37.000	42.33^{ab}	108^{efg}	86^{fg}	37.67^{abcd}
PusaAzotobacter liquid bio-fertilizer + *Amaranthusviridis*leaf extract	37.333	42^{ab}	114^{g}	74^{cdef}	39.67^{cde}
PusaAzotobacter liquid bio-fertilizer + *Echinochloacolona*leaf extract	37.667	49^{abcd}	105.33^{defg}	54^{abcd}	35^{ab}
PusaAzotobacter liquid bio-fertilizer + *Digeraarvensis*leaf extract	38.000	49.67^{abcd}	101.67^{bcde}	76.33^{defg}	39^{bcde}
Pusa PSB liquid bio-fertilizer + *Cyperusrotundus*leaf extract	38.000	53.33^{abcd}	103.33^{cdef}	101.33^{g}	37.67^{abcd}
Pusa PSB liquid bio-fertilizer + *Amaranthusviridis*leaf extract	36.667	67.33^{d}	112.67^{fg}	83^{efg}	42.33^{e}
Pusa PSB liquid bio-fertilizer + *Echinochloacolona*leaf extract	33.333	65^{cd}	107.33^{defg}	72.67^{cdef}	38.67^{bcde}
Pusa PSB liquid bio-fertilizer +*Digeraarvensis*leaf extract	35.000	60^{bcd}	97.33^{abcd}	77^{defg}	35.67^{abc}
Potash solublizing liquid bio-fertilizer+ *Cyperusrotundus*leaf extract	35.667	53.33^{abcd}	106.33^{defg}	63.67^{bcdef}	36.67^{abcd}
Potash solublizing liquid bio-fertilizer+ *Amaranthusviridis*leaf extract	35.000	46.33^{abcd}	106^{defg}	48^{abc}	39.67^{cde}
Potash solublizing liquid bio-fertilizer+ *Echinochloacolona*leaf extract	34.000	49.33^{abcd}	102.67^{bcdef}	42^{ab}	38^{abcd}
Potash solublizing liquid bio-fertilizer+*Digeraarvensis*leaf extract	33.667	38^{a}	91.67^{a}	34.33^{a}	38.33^{abcde}

Note: No significant difference between the treatments when same letter follows the mean in the same column (DMRT)

Table 3.
Influence of bio-fertilizers and weed leaf extracts (WLE) on Isabgol crop growth parameters at harvest stage

Treatment	Husk (g pot^{-1})	Seed (g pot^{-1})	Test weight
Control (sprayed with distilled water)	4.33^{abc}	9.33^{abcd}	2.039
Pusa zinc solublizingbio-fertilizer + *Cyperusrotundus*leaf extract	7.33^{de}	15.67^{de}	2.033
Pusa zinc solublizingbio-fertilizer + *Amaranthusviridis*leaf extract	3.33^{a}	5.67^{ab}	2.041
Pusa zinc solublizingbio-fertilizer + *Echinochloacolona*leaf extract	7.67^{de}	14^{cd}	2.041
Pusa zinc solublizingbio-fertilizer +*Digeraarvensis*leaf extract	6.33^{cde}	14.33^{d}	2.042
PusaAzotobacter liquid bio-fertilizer + *Cyperusrotundus*leaf extract	6.33^{cde}	11.67^{bcd}	2.040
PusaAzotobacter liquid bio-fertilizer + *Amaranthusviridis*leaf extract	8.33^{e}	16^{de}	2.051
PusaAzotobacter liquid bio-fertilizer + *Echinochloacolona*leaf extract	5^{abcd}	5.67^{ab}	2.016
PusaAzotobacter liquid bio-fertilizer + *Digeraarvensis*leaf extract	6.67^{cde}	14.67^{de}	2.041
Pusa PSB liquid bio-fertilizer + *Cyperusrotundus*leaf extract	7^{cde}	21.33^{e}	2.031
Pusa PSB liquid bio-fertilizer + *Amaranthusviridis*leaf extract	6^{bcde}	13.67^{cd}	2.039
Pusa PSB liquid bio-fertilizer + *Echinochloacolona*leaf extract	7^{cde}	14.67^{de}	2.046
Pusa PSB liquid bio-fertilizer +*Digeraarvensis*leaf extract	5.33^{abcd}	12^{bcd}	2.051
Potash solublizing liquid bio-fertilizer+ *Cyperusrotundus*leaf extract	5.33^{abcd}	14^{cd}	2.029
Potash solublizing liquid bio-fertilizer+ *Amaranthusviridis*leaf extract	6^{bcde}	7.33^{abc}	2.024
Potash solublizing liquid bio-fertilizer+ *Echinochloacolona*leaf extract	3.67^{ab}	6.33^{ab}	1.920
Potash solublizing liquid bio-fertilizer+*Digeraarvensis*leaf extract	3.33^{a}	3.67^{a}	1.996

Note: No significant difference between the treatments when same letter follows the mean in the same column (DMRT)

RESULTS AND DISCUSSION

Plant growth characteristics

Isabgol plant growth parameters during growth stages were not significantly influenced by all the treatments applied. Although plant height and number of tillers / plant was greater in all of the treatments as compared to control, in case of plant canopy all the treatments except Pusa zinc solubilizing bio-fertilizer along with *Digeraarvensisweed* leaf extract and Pusa Azotobacter liquid bio-fertilizer along with *Digeraarvensisweed* leaf extract and Potash solublizing liquid bio-fertilizer along with *Amaranthusviridisweed* leaf extract recorded higher plant canopy than control (Table 1). At harvest stage, Pusa PSB liquid bio-fertilizer along with *Amaranthusviridisweed* leaf extract gave significantly highest plant canopy (67.33 cm) and dry matter (42.33 g / pot) while lowest values for plant canopy and dry matter was recorded from Potash solublizing liquid bio-fertilizer along with *Digeraarvensisweed* leaf extract (38 cm) and control (34.33 g / pot) respectively. PusaAzotobacter liquid bio-fertilizer along with *Amaranthusviridisweed* leaf extract gave significantly highest number of spikes / plant (114) followed by Potash solublizing liquid bio-fertilizer along with *Digeraarvensisweed* leaf extract (112.67) while the lowest no. of spikes / plant were recorded from Potash solublizing liquid bio-fertilizer along with *Digeraarvensisweed* leaf extract (91.67). Pusa PSB liquid bio-fertilizer along with *Cyperusrotundusweed* leaf extract gave significantly highest fresh biomass (101.33 g / pot) followed by PusaAzotobacter liquid bio-fertilizer along with *Cyperusrotundusweed* leaf extract (86 g / pot) while lowest fresh biomass was recorded from Potash solublizing liquid bio-fertilizer along with *Digeraarvensisweed* leaf extract (34.33 g / pot) (Table 2). Increase in dry matter could be attributed to the increase in N, P, K content of the leaves which may have enhanced cell division and increased organic products biosynthesis which would have lead to the carbohydrate and protein accumulation in leaves. These results were in conformity with the findings of Anisuzzaman *et al.* (2014) and Yadegari *et al.* (2012) in Sennaobtusifolia and Garden Thyme respectively.

Seed and Husk Yield

Combined effect of bio-fertilizers and weed leaf extract showed significant impact on husk and seed yield of Isabgol plant. PusaAzotobacter liquid bio-fertilizer along with *Amaranthusviridisweed* leaf extract gave significantly highest husk / plant (8.33) followed by Pusa zinc solublizingbio-fertilizer along with *Echinochloacolonaweed* leaf extract (7.67) while lowest husk / plant was recorded from Potash solublizing liquid bio-fertilizer along with *Digeraarvensisweed* leaf extract (3.33) and Pusa zinc solublizingbio-fertilizer along with *Amaranthusviridisweed* leaf extract (3.33). Pusa PSB liquid bio-fertilizer along with *Cyperusrotundusweed* leaf extract gave significantly highest seed / plant (21.33) followed by PusaAzotobacter liquid bio-fertilizer along with Amaranthusviridis leaf extract (16) while lowest seed / plant was recorded by Potash solublizing liquid bio-fertilizer along with *Digeraarvensisweed* leaf extract (3.67). Besides direct physiological assistance, weed leaf extracts may have also acted as a topical green fertilizer, bio-stimulant or foliar feed which indirectly assisted in increasing seed yield (Jama *et al.* 2000, Shaaban 2001) and husk yield (Pretali *et al.* 2016). Due to the presence of major plant nutrients and beneficial soil microbes in the biostimulants (bio-fertilizers and weed leaf extracts), husk and seed yield of Isabgol enhanced majorly in the present study. Leguminous weed seeds like *Amaranthusviridisand Cyperusrotundus* contain high levels of N, P, K nutrients, and their extracts contain these nutrients in a form that can easily be taken up by the plants. Application of bio-fertilizers and weed leaf extracts together could have drastically improved soil processes as well. These findings were similar to the ones reported by Basak *et al.* (2020) in Ashwagandha and Sudhakara (2005) in *Thymus vulgaris.*

CONCLUSION

Weed plants can be effectively utilised as a source of green fertiliser for enhancing productivity of crops as an effective waste to wealth management approach. Synergistic effect of these extracts with microbial bioinoculantsmay be promising combination for achieving sustainable and eco-friendly nutrient management.

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