EFFECT OF EM, RHIZOBIUM AND PSM ALONE AND IN COMBINATION ON THE YIELD OF RADISH (***Raphanus sativus L.***)

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ABSTRACT
The study was conducted to evaluate the Effective microorganism (EM), *Rhizobium,* Phosphate solubilizing bacteria (PSB) used alone and in coinoculation on physiology and yield of radish (Raphanus sativus L.) with particular reference to the role of microbes in the production of phytohormones and antioxidant.
The experiment was conducted in pots in green house of Quaid-i-Azam University Islamabad under natural environmental condition. The plants were treated with effective microorganisms (EM), Bokashi, *Rhizobium leguminosarum*; strain Thal 8, Farm yard manure used alone and in combination.
Treatment with EM extended solution alone and more so in combination with Bokashi showed maximum number of leaves , maximum chlorophyll content, greater sugar content ,higher TSS,(total soluble solids),greater peroxidase activity and increased catalase activity. weight of edible radish, weight of seed as compared to uninoculated control. Plants inoculated with Rhizobium also showed increase in number of leaves, weight of radish and weight of seed. Over control (uninoculated) plants but the magnitude of stimulation was minimum. Simultaneous application of Farm yard manure and EM extended solution resulted in significant increase in growth and yield. The significant increase of IAA and GA content of leaves were obtained in all the treatments. The magnitude of stimulation was significantly greater following EM extended + Bokashi. Combined treatment of EM extended and Bokashi with farmyard manure is demonstrated for increased yield.

INTRODUCTION

Pakistan is gifted with varied agroclimatic condition for growing an array of vegetable crops. To eliminate malnutrition and to relieve the over stress on cereals, there is great need of growing vegetables. The total area under vegetable cultivated in Pakistan is 256.4 thousand hectares with 3568.8 thousand tons production (excluding potato and sugar beat) (Anonymous, 1997).
Radish (*Raphanus sativus* L.), commonly known, as "Mooli" is one of the most important vegetables grown for their edible freshly roots. Beside the effective control of pest and disease, crop fertilizer also plays an important role towards high return.
EM-Bokashi has the ability to attribute the biologically active substances along with the sustained release of available plant nutrients from the decomposition of organic materials (Yamada and XU, 2000).
Fruits and vegetables are good sources of many antioxidants, diets rich in these foods are associated with a lower risk of the chronic disease of cancer Hennekens(1986) and heart diseases (Vanpoppel et al.1994).
Catalase and peroxidase are the major antioxidant enzyme associated with scavenging the active oxygen species and are important defense against toxic substances. (Vang et al. 2001; Alscher et al. 2002).

The present attempt aims to investigate the effect of EM, Bokashi, PSM and Rhizobium alone and in combination on the biochemical content, catalase and peroxidase activity and yield of radish (*Raphanus sativus* L.).

Plant material and Growing condition

Seeds of radish (Raphanus sativus L.) cv. red round were obtained from National Agricultural Research Center, Islamabad. Experiment was conducted under natural environmental condition in the Department of Biological Sciences Quaid-I- Azam University, Islamabad. Earthern pots (30×40cm) were filled with mixture of sand and soil (1:3) Seeds were surface sterilized using 10% chlorox. Thereafter, planted into each pot to a depth of 3cm during mid November 2003. The solution of EM was obtained from EM Research Organization Japan. EM-Bokashi was prepared in phytohormone laboratory of Department of Biological sciences, Quaid-I-Azam University Islamabad following the method described (Daly,1998)

Method of Inoculation

Seeds were pelleted prior to sowing with the carrier (mineral soil) based inocula of *Rhizobium leguminosarum* strain TAL 620.

Following treatments were made:

Symbol	**Treatments**
C	Control (without inoculum)
E	Plants inoculated with EM extended inoculum
B	Plants inoculated with EM-Bokashi inoculum
R	Plants inoculated with Rhizobium
E+B	Plants inoculated with EM extended + Bokashi inoculum
F+E	Plants receiving Farm Yard Manure + EM extended inoculum

Plant Sampling

Plants were collected during vegetative phase, 90 days after sowing for determining chlorophyll, sugar, protein, catalase and peroxidase from leaves of radish.

Sugar estimation of fresh leaves was done by method of Dubo et al. (1956) as modified by Johnson et al. (1966).Protein estimation of fresh leaves was done by method of Lowry et al. (1951)Chlorophyll content was measured by Arnon (1949) modified by Krick (1968)

Peroxidase activity of leaves was measured by method of Vetter et al (1958) and Catalase activity of leaves was measured by Arnon (1985). Total soluble solid was determined by using the hand refractometer.

The extraction and purification for plant hormones from leaves IAA and GA was made following the method of Kettner and Doerffling (1995). The analyses for IAA and GA were made by HPLC using variable UV detector following the method of Li *et al*. (1994).

Statistical analysis

The data so collected were subjected to analysis of variance technique (Steel and Torrie, 1985) and treatment means were compared by using Duncan's Multiple Range Test (Duncan, 1961).

Table 11 Effect of different microorganisms alone and in combination on yield parameters of radish leaves. Measurements were made (90 days after sowing) at harvest. The treatments were applied at time of sowing .E and B treatments receive dilute solution of EM extended (1:1000) for irrigation throughout the experiment till harvest of the plant.

Treatment	* Number of leaves	** Fresh weight (g)	*** Dry weight(g)
C	7.000c	75.00e	7.00c
E	12.00ab	128.0c	12.00ab
B	12.00ab	126.0c	12.00ab
E+B	14.00a	140.0a	12.75ab
F+E	13.25a	134.0b	13.25a
R	10.00b	110.0d	9.75bc

Column means followed by a common letter are not significantly different at 5%.

*** LSD Value = 2.991**
**** LSD Value = 5.651**
***** LSD Value = 3.037**

Table 12 Effect of different microorganisms alone and in combination on yield parameters of radish. Measurements were made (120 days after sowing) at harvest. The treatments were applied at time of sowing. In which case E and B treatment dilute solution of EM extended (1:1000) was used for irrigation throughout the experiment till harvest of the plant.

Treatment	* Number of beans	** Weight of bean(g)	*** Number of seed	**** Weight of seed(g)	***** Fresh weight of radish(g)
C	14.00d	32.00e	80.00e	30.00d	98.00e
E	22.00c	73.00bc	120.0c	70.00b	156.0b
B	21.00c	69.00c	117.0c	68.00b	144.0c
E+B	31.00a	94.25a	144.5a	90.00a	198.0a
F+E	26.00b	75.00b	135.0b	72.00b	160.0b
R	20.00c	48.00d	105.0d	41.00c	130.0d

Column means followed by a common letter are not significantly different at 5%.

*** LSD Value = 3.973**
**** LSD Value = 4.081**
***** LSD Value = 6.282**
****** LSD Value = 5.684**
******* LSD Value = 7.296**

Table 13 Effect of different microorganisms alone and in combination on biochemical contents of radish leaves. Measurements were made (90 days after sowing) at harvest. The treatments were applied at time of sowing. In which case E and B treatment dilute solution of EM extended (1:1000) was used for irrigation throughout the experiment till harvest of the plant.

Treatment	* Chlorophyll	** Sugar	*** Protein	**** TSS
C	36.00c	2.00d	5.175d	2.950d
E	46.00b	6.00c	9.375b	4.100c
B	52.00a	7.20b	9.675b	4.125c
E+B	53.00a	9.25a	12.40a	7.300a
F+E	47.00b	8.00b	9.675b	6.300b
R	45.00b	6.00c	7.300c	3.950c

Column means followed by a common letter are not significantly different at 5%.

*** LSD Value = 2.968**
**** LSD Value = 0.8774**
***** LSD Value = 0.3856**
****** LSD Value = 0.3769**

Table 14 Effect of different microorganisms alone and in combination on biochemical contents of radish leaves. Measurements were made (90 days after sowing) at harvest. The treatments were applied at time of sowing. In case of E and B treatment, dilute solution of EM extended (1:1000) was used for irrigation throughout the experiment till harvest of the plant. The activity of catalase and peroxidase were expressed as % of the control assuming control as 100%.

Treatment	* IAAµg/g	** GAµg/g	*** PODµg/g	**** CATµg/g
C	164.5f	6.60f	100f	100f
E	372.8c	13.75b	172d	206c
B	315.8d	12.00c	211c	312a
E+B	592.0a	15.75a	268a	249b
F+E	506.0b	11.00d	248b	105d
R	224.8e	9.25e	71e	48e

Column means followed by a common letter are not significantly different at 5%.

*** LSD value = 34.28**
**** LSD Value = 0.7985**
***** LSD value = 0.5805**
******** LSD Value = 0.2151

Table 15 Effect of different microorganisms alone and in combination on soil parameters of radish. Measurements were made (120 days after sowing) at harvest. The treatments were applied at time of sowing. In which case E and B treatment dilute solution of EM extended (1:1000) was used for irrigation throughout the experiment till harvest of the plant.

Treatment	* Soil pH	** ECE		**** K	***** P
C	7.833a	1.233a		161.7c	3.717e
E	7.33c	0.3833b		236.7b	7.100b
B	7.300cd	0.4167b		236.7b	6.707c
E+B	7.133d	0.3667b		251.7a	7.940a
F+E	7.300cd	0.4333b		252.0a	7.177a
R	7.567b	0.5500b		164.00c	6.440d

Results

Number of leaves and Fresh and Dry weight of leaves

Result (Table 1) demonstrated that number of leaves and fresh and dry weight of leaves were significantly increased in all the treatments as compared to control. The magnitude of stimulation was maximum due to combined effect of EM, Bokashi and FYM.

Rhizobium showed least increase among all the treatments. Single inoculation of EM extended and Bokashi showed less increase than that of combined treatments, the dry weight was maximum with the combined treatment of EM with Farm yard manure.

Number and weight of bean and weight of seed per plant

Data (Table 2) demonstrated that number and weight of bean and seed increased significantly in all the treatments over control. The least increase in number and weight of seed was observed following inoculation with *Rhizobium*. Simultaneous application of EM extended and Bokashi showed synergism in action exhibiting maximum increase in number and weight of bean and seed and fresh weight of radish. Whereas, combination of E with FYM showed relatively greater increase in weight but less increase in number of bean, indicating a compensatory mechanism of more increase in bean size with fewer beans produced.

Chlorophyll, sugar, protein, total soluble solid and bio mass of radish

The data (Table 3) showed the significant increase of chlorophyll, sugar, protein, total soluble solid content in all the treatments over control. The combined effect of EM and Bokashi (E+B) was more favorable as compared to single treatments as well as other treatments. Minimum increase was observed in *Rhizobium* inoculated plants.

IAA and GA concentration and peroxidase and catalase activity of leaves

Data (Table 4) demonstrated the significant increase of IAA and GA content among all the treatments. The magnitude of stimulation was significantly greater following EM extended + Bokashi. The combined application of FYM + EM extended treatment was more stimulatory over the single application of E with respect to the IAA content whereas, the increase in GA content was more in single treatment of E. Minimum increase was recorded in *Rhizobium* inoculated plants.

The concentration of peroxidase increased significantly among all the treatments. The combined treatment of EM Extended + Bokashi was more effective than E or B treatments made alone. Bokashi treatment resulted in the maximum increase in the activity of catalase followed by combined effect of EM extended + Bokashi. It was observed that catalase concentration in radish leaves was less than that of peroxidase.

Soil pH, ECe and P, K content of soil

Data presented in Table 15 showed that soil pH previously cultivated with radish was decreased in the combined treatments with EM extended and Bokashi. Co-inoculation with E+B revealed marked decrease in soil previously cultivated with radish. *Rhizobium* treated plants showed decrease pH as compared to control. Soil previously cultivated with E+B showed further decrease in the ECe of soil as compared to that of *Rhizobium* treated plants.

Potassium and Phosphorous content was increased significantly in all the treatments as compared to control. Maximum effect was observed when combined treatment of EM extended + Bokashi and FYM + EM extended were applied.

DISCUSSION

During the present investigation, chlorophyll content was increased significantly alone and in combination with EM extended and Bokashi treatments. This may be due to synergistic effect as reported earlier (Yamada and XU, 2000) that stimulation in root growth from added Bokashi may also help to maintain a higher rate of growth and photosynthetic activity. Result showed that chlorophyll and protein content and enzyme level was significantly increased alone and in combination with EM extended and Bokashi treated plants. Minsk (1998) also reported the increased level of protein, chlorophyll and peroxidase enzyme following EM and Bokashi treatments. Harward,(2000) reported that application of EM has increased the beneficial type of

microorganism such as *Rhodobacter, Pseudomonas, Lactobacillus,* and *Gluconobacter* etc, which have ability to produce protein, mineral and antioxidants in soil. Combination of EM and Bokashi significantly increased yield as reported by Jamil., et al (1995) that application of EM Bokashi increased the number of *Azotobacter, Azospirillum, Bacillus* and *Lactobacillus* in the soil, subsequently increased yield parameters. Saikander (1998) found that bacterial count was more than double to FYM than urea. Sangakkara, (1994) reported that EM enhanced germination, plant growth, leaf area and increased yield component. EM increased the pod number, pod weight and seed yield as reported by Hussain et al., (1994) and Chingfang et al., (1995). Co-inoculation effect of rhizobial strain was noted to be higher than single strain application and this may be due to synergistic effect as reported by Camacho et al, (2001) and Jain et al. (1999).

CONCLUSION

It is inferred from the present finding that EM and Bokashi can be used to significantly increase the antioxidants as obvious from the EM or Bokashi induced increase in catalase and peroxidase concentration. The EM effect is further augmented in presence of Farm yard manure applied to soil.

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