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RESEARCH ARTICLE

Comparative Effects of Biofertilizers, Chemical Fertilizer and Fungicide on Growth of Brassica nigra

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Abstract

The present study examined the outcome of biofertilizers, chemical fertilizer and fungicide alone and in combinations on growth and biochemical parameters of *Brassica nigra* at the 30th and 60th day of germination. Result showed significant enhancement in growth and biochemical parameters of plants as compared to control plants. Trichoderma hamatum and rhizobial isolates treatments alone and in combination with each other and with chemical fertilizer were found effective in improving the shoot and root lengths, total chlorophyll, carbohydrate, crude protein, and mineral (nitrogen and phosphorus) content of *Brassica nigra*. It indicates that biofertilizers alone and in combination enhanced total carbohydrate, protein and nitrogen contents may boost the soil fertility by improving its organic and inorganic content. Therefore, biofertilizers could be a good alternate of chemical fertilizer and fungicide for improving the growth and productivity of *Brassica nigra.*

Introduction

In Pakistan, here are a lot of causes that limit the sustainable development of crops as well as soil salinity, alkalinity, erosion, minimum water availability, shortage of agricultural land and improper practices; all these severely affect the soil fertility (Bhutto & Bazmi, 2007). To resume the fertility of soil and stimulate the agriculture productivity, chemical fertilizers are the first choice of farmers, though; their consecutive, excessive and inappropriate use can cause some health and environmental risks (Smith et al., 2008). Beside these environmental hardships, plant pathogens are another serious threat to agriculture like fungi are considered as unsupportive agents and reported for serious losses of crop yield, value and income (Keane,2012; González-Fernández et al.,2010) which can be prevented by chemical fungicides globally (Dias,2012).

Now-a-days, biotechnologists are finding few alternatives of these synthetic fertilizers and fungicides and constantly making efforts to create an extra beneficial soil position for outstanding crop production and defense during controlling and manipulating the soil micro-flora through biofertilizers, organic modifications and cultural & management practices (Laditi,2012). Biofertilizers are really a combination of potentially dynamic live microorganisms (bacteria or fungi) which directly or indirectly effect the crop development and yield through number of methods (Ahemad & Khan, 2011; Pandya & Saraf, 2010). In addition, use of biofertilizers also reduces the chances of environmental hazards and increases the stability of soil ecosystem (Mia & Shamsuddin , 2010). Studies reported that better the diversity and quantity of microbial residents, the high is the order of their contact by means of plant roots and the additional established the ecosystem (Nihorimbere et al., 2010). In this regard, endophytic microorganisms both bacteria and fungi provide both growth stimulators and biocontrol agents

(Kaewchai et al., 2009). After observing the growth promoting effects of *Trichoderma hamatum* alone and in combination with *rhizobium* and *bradyrhizobium* species on sunflower and mash beans. The present study was designed to compare the effects of these microorganisms with chemical fertilizer and fungicide on growth of *Brassica nigra* (black mustard) L.

Brassica nigra (black mustard) L., belongs to the family *Brassicaceae*, is a quick growing annual herb and show water absorbing capacity by growing its tap roots 5 feet deep into the soil (Shekhawat et al.,2012). It has variety uses like oil of this mustard used for food preparation in many areas of India (Piri,2012), medicinally its ground seeds are used as appetizer, digestive and diuretic agents while with honey use to release cough and respiratory infections (Hassan, 2006). The leaves, stem, pods and husk of this plant are reported to use as feed for livestock, cover crop and green manure in agriculture and also serves as a supply of biodiesel (Bannikov, 2011).

Material and Methods

Use of microbial inoculants (as biofertilizers) including *T.hamatum*, *rhizobium* and *bradyrhizobium* isolates alone and in grouping, NPK (as chemical fertilizer)and carbendazim (as chemical fungicide) were used to examine their outcome on growth and biochemical parameters of experimental plants (Table 1).

Conidial and cell inoculums of *T.hamatum* and rhizobial isolates were prepared for conducting pot experiments to investigate the effect of microbial inoculants on growth and biochemical parameters of Brassica nigra plants. For this serial dilutions from 1:100 to 1:10,000 were made. Twenty five milliliters of highest dilution was used as inoculum after adjusted the concentration about 1.2×10^{6} cfu/ml (for T.hamatum) with the help of SMIC haemocytometer ART. No.1280. Similar procedure was used to prepare cell inoculums of rhizobial isolates, calculated and adjusted to 1.9×10^{8} cfu/ml (Tuite, 1969; Badar & Qureshi,2012).

The randomized complete block designed pot experiment was conducted in net house of Department of Botany, Jinnah University for Women, Nazimabad, Karachi, Pakistan to investigate the growth and biochemical parameters of experimental plants. Seeds of experimental plants were sown in pots filled with 2 kg soil in each. On 5th day of germination, developing seedlings in each pot of each block were inoculated with twenty five milliliters of its respective treatment. Five replicates were used for each treatment. Five plants of each treatment (1 plant/replicate/treatment) were uprooted at 30th and 60th day of growth to measure the selected physical and biochemical parameters. Similarly five pots treated with each of NPK and carbendazim @ 2500 ppm and were used as positive controls while other five pots of experimental plants without any treatment were used as control.

Root length; shoot length and plant fresh weight (biomass) was recorded as physical parameter. For biochemical parameters total chlorophyll & its fractions (a, b) and total carbohydrate contents were determined by methods described by Arnon and Yemm & Willis (Arnon, 1949; Yemm & Willis, 1956) while crude protein by multiplying percent nitrogen value through 6.25 (Sriperm et al., 2011). The mineral content (percent nitrogen and phosphorus) were estimated by Nessler's (Singh, 1982) and Barton reagent (Ashraf et al., 1992).

The data was analyzed by using *One-way* ANOVA followed by LSD (least significant difference) test through SPSS 16. The differences were considered significant at $p<0.05$ when treatments' mean compared with control. Results of present pot experiments are expressed as mean ± standard deviation (S.D.).

Results

Growth performance

T.hamatum (JUF1) alone induced less than 50% enlarge in root and shoots lengths of mustard plants at both 30th and 60th days. The same positive effects were observed in groups of plants treated with JUF1 in combination with JUR3 at both days of harvesting. (Table 2). All rhizobial isolates promote the root length from 22-26% of test plants at both days while shoot length from 27-66% at 30th day. JUR3+FTZ increased root length from 28-45% at both days and shoot length 51% at 30th day while JUR4+FTZ induced increase in both of these physical parameters from 16-34% at both days. Along with FGD, JUR4 found more efficient in increasing root length from 19-22% at both days of uprooting of plants as compared to JUR3 + FGD (Table 2).

T.hamatum alone improved fresh weight of mustard plants at 60th day (Table 2). Rhizobial isolates JUR3 and JUR4 induced increase in fresh weight of test plants with 179 and 86% respectively at 30th day while JUR3 also

increased the same parameter with 30% at 60th day. In combination with FTZ, JUR3 improved the fresh weight of test plants at 30th day with 62% while JUR3+FTZ also produced 37% improvement in fresh weight at 60th day (Table 2).

Photosynthetic pigment

T.hamatum (JUF1) alone and co-inoculated with JUR3 and JUR4 increased total chlorophyll and its fractions from 16-105% in leaves of mustard plants at 30th day while the same treatments promoted the total chlorophyll and its fractions from 35-132% at 60th day. While JUF1 improved photosynthetic pigments from 35-61% at 60th day in combination with FTZ. (Table 3).

Out of rhizobial isolates, JUR3 found better in promoting the synthesis of total chlorophyll (66%) and its fractions (102-131%) in test plants at 30th day and while chl-b (112%) at 60th day, followed by JUR4 promoted the chl-a (78%) at 30th day and chl-b (84%) at 60th day. In combination with FTZ, JUR3 and JUR4 enhanced the synthesis of total chlorophyll with its fractions from 15-68% at 30th day. Whereas improved fraction a, b and total chlorophyll from 21-95% at 60th day in test plants. (Table 3).

Biochemical parameters

T.hamatum (JUF1) alone increased carbohydrate content with 215% in mustard plants at 30th day. The same fungus co-inoculated with JUR3 and JUR4 significantly stimulated the carbohydrate synthesis in leaves of test plants from 113-195%

at both days of harvesting of plants. Similarly JUR3 (119-129%) and JUR4 (146-240%) individually in their respective group improved the carbohydrate amount at 30th and 60th day. In combination with FTZ, JUR3 found most effective in increasing the carbohydrate content from 120-140% at both days, followed by JUR4+FTZ with 33% at 30th day and 150% increase at 60th day. Along with FGD, JUR3 and JUR4 found effective in increasing the amount of total carbohydrate from 150-210% at 30 day and from 79-165% at 60th day. (Table 4).

T.hamatum alone improved crude protein content with 87% in test plants and co-inoculated with JUR3 and JUR4 enhanced 78-184% at 30th day while 116-129% at 60th day. JUR4 found effective in enhancing the crude protein content with 136-241% in test plants at both days, followed by JUR3 with 109% at 30th day. JUR3 and JUR4 with FTZ improved the same parameter from140-150% at 60th day. In case with fungicide (FGD), JUR3 and JUR4 increased crude protein content with 112 and 165% respectively at 30th and 60th day (Table 4).

Mineral content

T.hamatum (JUF1) alone increased percent nitrogen in mustard plants at 30th day. The same fungus co-inoculated with JUR3 promoted the nitrogen content 186% and 130% respectively at 30th and 60th day. JUF1+JUR4 80% increased the same mineral content at 30th day and 117% at 60th day. Out of two rhizobial isolates, JUR4 found most efficient in increasing the nitrogen content of test plants from 139-242% at both days, followed by JUR3 with 111% at 30th day. In combination with FTZ, JUR3 and JUR4 induced increase from 141-152 % in nitrogen content of test plants at 60th day. Whereas JUR3 with FGD increased the same parameter 114% at 30th day and JUR4+ FGD 166% at 60th day (Table 5).

T.hamatum co-inoculated with JUR3 and JUR4 improved the phosphorus content in mustard plants respectively 106% at 30th day and 114% at 60th day. Rhizobial isolates include JUR3 and JUR4 significantly increased phosphorus content in test plants from 75-114% at both days of uprooting of plants (Table 5).

Table 1: Treatments of test microbial inoculants alone and in combination used in experiment

S.no.	Treatment	Code
1.	Control	Control
2.	*Trichoderma hamatum* (1.2 x 10^{6} cfu/ml)	JUF1
3.	*Bradyrhizobium specie*-III (1.9 x 10^{8} cfu/ml)	JUR3
4.	*Bradyrhizobium specie*-IV(1.9 x 10^{8} cfu/ml)	JUR4
5.	Fertilizer (NPK @ 2500 ppm)	FTZ
6.	Fungicide (Carbendazim @ 2500 ppm)	FGD
7.	*Bradyrhizobium specie* -III (1.9 x 10^{8} cfu/ml) + *T. hamatum* (1.2 x 10^{6} cfu/ml)	JUR3 + JUF1
8.	*Bradyrhizobium specie* -IV (1.9 x 10^{8} cfu/ml) + *T. hamatum* (1.2 x 10^{6} cfu/ml)	JUR4 + JUF1
9.	*Bradyrhizobium specie* -III (1.9 x 10^{8} cfu/ml) + NPK (2500 ppm)	JUR3 + FTZ
10.	*Bradyrhizobium specie* -IV (1.9 x 10^{8} cfu/ml) + NPK (2500 ppm)	JUR4 + FTZ
11.	*Bradyrhizobium specie* -III (1.9 x 10^{8} cfu/ml) + carbendazim (2500 ppm)	JUR3 + FGD
12.	*Bradyrhizobium specie* -IV (1.9 x 10^{8} cfu/ml) + carbendazim (2500 ppm)	JUR4 + FGD
13.	*Trichoderma hamatum* (1.2 x 10^{6} cfu/ml) + NPK (2500 ppm)	JUF1 + FTZ

Table: 2 Effect of treatments on growth performance of *B.nigra* plants

S. No	Treatment	Growth performance 30th days Root length*		Shoot length*		Fresh weight**		60th days Root length*		Shoot length*		Fresh weight**	
1	Control	9.84 ± 0.50		13.74 ± 0.90		0.41 ± 0.04		23.70 ± 0.57		31.66 ± 0.57		4.72 □ 0.24	
2	JUR3	12.06 ± 1.47[c]	(23)	22.83 ±1.15[a]	(66)	1.17 ± 0.14[a]	(179)	29.10 ±1.38b	(23)	35.66 ± 2.51	(13)	6.15 □ 1.77[d]	(30)
3	JUR4	12.00 ±1.44[c]	(22)	17.50 ±0.90[d]	(27)	0.78 ± 0.13[c]	(86)	29.83 ± 1.15[a]	(26)	33.46 ± 1.17	(6)	5.36 □ 0.12	(14)
4	JUF1	11.66 ± 0.28[d]	(19)	18.00 ±1.35[c]	(31)	0.53 ±0.08	(26)	31.50 ± 3.12[a]	(33)	38.33 ±4.04[c]	(21)	6.82 □ 0.46[b]	(45)
5	FTZ	12.2 ±1.25[b]	(24)	16.90 ±1.21[d]	(23)	0.54 ± 0.10	(29)	28.43 ± 1.70[c]	(20)	37.00 ± 5.56[d]	(17)	5.05 □ 1.01	(7)
6	FGD	11.13 ± 0.96	(13)	15.03 ± 0.80	(9)	0.53 ± 0.83	(26)	28.40 ± 1.73[c]	(20)	29.33 ± 0.57	(-7)	2.77 □ 1.05	(-41)
7	JUR3+JUF1	11.36 ±1.50[d]	(16)	17.13 ±0.65[d]	(25)	0.47 ± 0.04	(12)	30.50 ± 3.12[a]	(29)	36.00 ± 2.64[d]	(14)	5.18 □ 0.57	(10)
8	JUR4+JUF1	11.16 ± 0.61	(17)	17.93 ±3.53[c]	(31)	0.44 ± 0.03	(5)	31.06 ± 0.75[a]	(31)	35.56 ± 0.77	(12)	5.24 □ 0.04	(11)
9	JUR3+FTZ	12.6 ± 0.55[b]	(28)	20.83 ±1.21[a]	(52)	0.68 ± 0.15[d]	(62)	34.40 ± 2.15[a]	(45)	28.33 ± 3.21	(-11)	6.46 □ 0.51[c]	(37)
10	JUR4+FTZ	11.46 ± 0.60	(17)	16.30 ± 3.35	(34)	0.56 ± 0.20	(33)	30.53 ± 0.83[a]	(29)	36.8 ± 1.05[d]	(16)	4.99 □ 0.52	(6)
11	JUR3+FGD	11.33 ± 0.70	(15)	14.76 ± 0.30	(8)	0.36 ± 0.02	(-14)	28.46 ± 0.96[c]	(20)	34.66 ± 0.57	(9)	4.79 □ 0.65	(1)
12	JUR4+FGD	11.73 ± 0.37[d]	(19)	15.73 ± 0.98	(15)	0.45 ± 0.12	(7)	28.90 ± 1.15[b]	(22)	32.23 ± 2.63	(2)	4.61 □ 0.57	(-2)
13	JUF1+FTZ	10.3 ± 0.40	(5)	10.56 ± 2.69	(-23)	0.29 ±0.10	(-31)	29.66 ± 0.35[a]	(25)	34.66 ± 1.52	(9)	4.81 □ 0.69	(2)

Each value is the mean ± S.D (standard deviation) of 5 replicates. Means bearing superscripts in each column are significantly different with respective control at $p < 0.05$(LSD). Values within parenthesis represent precent increase or decrease (-) with respective control. * = cm, ** = gm

Table 3: Effect of treatments on photosynthetic pigment of *B.nigra* (black mustard) plants

		Photosynthetic pigment											
		30th days						60th days					
S.No	Treatment	Chl-a*		Chl-b*		Total Chl *		Chl-a*		Chl-b*		Total Chl*	
1	Control	0.73 ± 0.03		0.57 ± 0.07		1.67 ± 0.05		0.77 ± 0.03		0.43 ± 0.04		0.93 ±0.08	
2	JUR3	1.69 ± 0.49[a]	(132)	1.15 ± 0.12[c]	(102)	2.78 ± 0.52[b]	(66)	0.78 ± 0.10	(1)	0.91 ± 0.21[a]	(112)	1.23 ± 0.39	(59)
3	JUR4	1.30 ± 0.38[c]	(78)	0.96 ± 0.04	(68)	2.17 ± 0.44	(30)	0.71 ± 0.05	(-8)	0.79 ± 0.03[c]	(84)	0.80 ± 0.20	(-32)
4	JUF1	1.45 ± 0.13[b]	(99)	0.93 ± 0.02	(63)	2.55 ±0.20[c]	(53)	1.36 ± 0.23[c]	(77)	0.74 ±0.48[c]	(72)	2.09 ± 0.44[a]	(125)
5	FTZ	0.92 ± 0.13	(26)	1.14 ± 0.47[c]	(100)	1.17 ± 0.89	(-30)	0.95 ± 0.03	(23)	0.74 ± 0.12[d]	(72)	1.05 ± 0.34	(13)
6	FGD	0.72 ± 0.09	(-1)	0.84 ± 0.12	(47)	0.93 ± 0.04	(-44)	1.28 ±0.24[c]	(66)	0.90 ± 0.09[a]	(109)	2.05 ± 0.27[a]	(120)
7	JUR3+JUF1	1.46 ± 0.34[b]	(100)	1.17 ± 0.21[c]	(105)	2.64 ± 0.56[c]	(58)	1.04 ± 0.40	(35)	1.00 ± 0.15[a]	(133)	1.49 ± 0.05[d]	(60)
8	JUR4+JUF1	0.85 ± 0.14	(16)	0.82 ± 0.06	(44)	1.68 ± 0.20	(0.6)	0.77 ± 0.97	(0)	0.87 ±0.06[b]	(102)	0.87 ± 0.23	(-6)
9	JUR3+FTZ	1.03 ±0.18	(41)	0.93 ± 0.06	(63)	2.1 ± 0.19	(26)	1.19 ± 0.40[d]	(55)	0.84 ± 0.13[b]	(95)	1.13 ± 0.35	(21)
10	JUR4+FTZ	1.65 ± 0.41[a]	(126)	0.93 ± 0.04	(63)	2.82 ± 0.61[b]	(69)	0.62 ± 0.02	(-19)	0.71 ± 0.05[d]	(65)	0.77 ±0.06	(-17)
11	JUR3+FGD	0.84 ± 0.06	(15)	0.69 ± 0.13	(21)	1.44 ± 0.29	(-14)	0.98 ±0.21	(27)	0.54 ± 0.08	(26)	1.53 ± 0.28[d]	(65)
12	JUR4+FGD	1.21 ± 0.02[d]	(66)	0.94 ± 0.33	(65)	2.02 ±0.24	(21)	0.66 ± 0.01	(-14)	0.68 ± 0.60[d]	(58)	0.75 ± 0.22	(-19)
13	JUF1+FTZ	0.67 ±0.04	(-8)	0.47 ± 0.19	(-18)	0.98 ± 0.4	(-41)	1.04 ± 0.50	(35)	0.68 ± 0.17[d]	(58)	1.50 ± 0.61[d]	(61)

Each value is the mean ± S.D (standard deviation) of 5 replicates. Means bearing superscripts in each column are significantly different with respective control at $p < 0.05$(LSD). Values within parenthesis represent precent increase or decrease (-) with respective control.* = mg/g, , chl = chlorophyll

Table 4: Effect of treatments on biochemical parameters of *B.nigra* (black mustard) plants

S. No.	Treatment	Biochemical parameters							
		30th days				60th days			
		Total carbohydrate (mg/g)		Crude protein (%)		Total carbohydrate (mg/g)		Crude proteins (%)	
1	Control	83.91± 3.98		4.05 ± 0.30		112.91 ± 91.00		5.21 ± 0.13	
4	JUR3	183.56 ± 46.10[b]	(119)	8.48 ± 2.13[c]	(109)	258.75 ± 18.14[d]	(129)	8.41 ± 2.76	(61)
5	JUR4	206.86 ± 14.97[a]	(147)	9.56 ± 0.69[a]	(136)	384.20 ± 100.11[a]	(240)	17.76 ± 4.63[a]	(241)
6	JUF1	264.87 ± 31.84[a]	(216)	7.57 ± 3.05[d]	(87)	188.11 ± 11.94	(66)	8.69 ± 0.55	(67)
7	FTZ	107.31 ± 10.14	(28)	4.96 ± 1.47	(22)	247.82 ± 126.30[d]	(119)	11.45 ±5.83	(120)
8	FGD	153.39 ±48.32[d]	(83)	7.09 ± 2.23[d]	(75)	172.99 ± 54.55	(53)	7.99 ± 2.52	(53)
11	JUR3+JUF1	248.24 ± 6.40[a]	(196)	11.48 ± 0.29[a]	(183)	258.44 ± 27.38[d]	(129)	11.95 ±1.26[d]	(129)
12	JUR4+JUF1	179.31±18.48[b]	(114)	7.21 ± 1.28[d]	(78)	243.96 ± 30.86[d]	(116)	11.28 ± 1.42	(117)
15	JUR3+FTZ	184.96 ± 7.00[a]	(120)	5.93 ± 1.13	(46)	270.64 ± 203.33[d]	(140)	12.51 ± 9.39[d]	(140)
16	JUR4+FTZ	111.96 ±3.38	(33)	5.17 ± 0.14	(28)	282.64 ± 40.02[d]	(150)	13.06 ± 1.85[d]	(151)
19	JUR3+FGD	260.27 ± 22.13[a]	(210)	8.59 ± 2.20[c]	(112)	202.27 ± 33.10	(79)	9.35 ± 1.52	(79)
20	JUR4+FGD	210.15 ± 17.72[a]	(150)	6.79 ± 0.73	(68)	299.44 ±54.73[c]	(165)	13.84 ± 2.53[c]	(166)
21	JUF1+FTZ	108.95 ±15.74	(30)	5.03 ± 0.72	(24)	130.62 ± 83.84	(16)	6.03 ± 3.87	(16)

Each value is the mean ± S.D (standard deviation) of 5 replicates. Means bearing superscripts in each column are significantly different with respective control at $p < 0.05$(LSD). Values within parenthesis represent percent increase or decrease (-) with respective control.

Table 5: Effect of treatments on mineral content of *B.nigra* (black mustard) plants

S. No.	Treatment	Mineral content 30th days Nitrogen (%)		Phosphorus (%)		60th days Nitrogen (%)		Phosphorus (%)	
1	Control	0.64 ± 0.05		0.16 ± 0.01		0.83± 0.02		0.21± 0.03	
4	JUR3	1.35 ±0.34[c]	(111)	0.28 ± 0.06[d]	(75)	1.34 ± 0.44	(61)	0.38 ± 0.11[d]	(81)
5	JUR4	1.53 ±0.11[a]	(139)	0.30 ± 0.08[d]	(88)	2.84 ± 0.74[a]	(242)	0.45 ± 0.08[c]	(114)
6	JUF1	1.21 ± 0.49[d]	(89)	0.16 ± 0.02	(0)	1.39 ± 0.90	(67)	0.19 ± 0.23	(-10)
7	FTZ	0.79 ± 0.07	(23)	0.11 ±0.01	(-31)	1.83 ± 0.93	(121)	0.14 ± 0.95	(-33)
8	FGD	1.13 ± 0.35[d]	(77)	0.15 ± 0.05	(-6)	1.27 ±0.40	(53)	0.15 ± 0.00	(-29)
11	JUR3+JUF1	1.83 ± 0.04[a]	(186)	0.33 ± 0.13[c]	(106)	1.91 ± 0.19[d]	(130)	0.24 ± 0.22	(14)
12	JUR4+JUF1	1.15 ±0.20[d]	(80)	0.11 ± 0.03	(-31)	1.80 ± 0.22	(117)	0.45 ± 0.20[c]	(114)
15	JUR3+FTZ	0.94 ±0.17	(47)	0.20 ±0.02	(25)	2.00 ± 1.50[d]	(141)	0.25 ± 0.10	(19)
16	JUR4+FTZ	0.82 ± 0.02	(28)	0.14 ± 0.05	(-13)	2.09 ± 0.29[d]	(152)	0.20 ± 0.13	(-5)
19	JUR3+FGD	1.37 ±0.35[c]	(114)	0.12 ± 0.73	(-25)	1.49 ± 0.24	(80)	0.21 ± 0.11	(0)
20	JUR4+FGD	1.08 ± 0.11	(69)	0.10 ± 0.17	(-38)	2.21 ± 0.40[c]	(166)	0.12 ± 0.04	(-43)
21	JUF1+FTZ	0.8 ± 0.12	(25)	0.16 ± 0.76	(0)	0.96 ± 0.62	(16)	0.28 ± 0.11	(33)

Each value is the mean ± S.D (standard deviation) of 5 replicates. Means bearing superscripts in each column are significantly different with respective control at $p < 0.05$(LSD). Values within parenthesis represent precent increase or decrease (-) with respective control.

Discussion

Black mustard (*B.nigra*) is one of the oilseed crops of *Brassicaceae* family; its seeds approximately contain 30-40% oil (Shekhawat et al., 2012). In the present study, inoculation of *T.hamatum* (JUF1) and rhizobial isolates (JUR3 & JUR4) alone and in combination significantly enhanced the growth parameters particularly root and shoot lengths of test plants.

The obtained positive effects of rhizobial isolates alone and in combination with *T.hamatum* on growth parameters of black mustard plants in present study support the previous reports that described the abilities of *rhizobium* strains in producing phytohormones in response to their inoculation *via* seed dressing or root drench which helped to speed up the growth and production of non-legumes (Sessitsch, 2002). A study proved that the direct stimulatory effect of *R.leguminosarum* inoculation on roots of *B.campestris* (another species of *Brassica*) and lettuce was found by producing indole-3-acetic acid and cytokinin, the growth regulators or phytohormones (Noel et al., 1996). Our study also proved that inoculation of *T.hamatum* with fertilizer or rhizobial isolates with each of fertilizer and fungicide produced beneficial effects on growth and biochemical parameters of non-legume plants. It has also been strengthen by evidences that described the bacterial inoculation promoted the plant growth by increasing N uptake and reducing the amount of nitrogen fertilizer that normally used (Mia & Shamsuddin, 2010). Chlorophyll content indicates the normal photosynthetic function of plant tissues which results in the formation of high energy-producing compounds in the presence of sunlight which are needed by plant for its regular metabolism. It has been reported that increased in chlorophyll content also linked to increase in total carbohydrate in plant tissues (Densilin, 2010), the same theme was achieved in our present study. On the other hand, increased protein content in growing parts of plant reflects the metabolic regulation associated with enhanced enzyme activity which helps plant to withstand environmental conditions and to promote their growth (Patil, 2010).

The growth promoting effects observed by *T.hamatum* alone and in combination with rhizobial isolates was confirmed its ability to produce antibiotics in rhizosphere that restrict the growth of microorganisms which have detrimental effects on plant growth (Kaewchai et al., 2009; Mohiuddin et al., 2010).Recently strain 382 of *T. hamatum* reported to reduce the occurrence of foliar diseases of several vegetable crops including tomato by altering genes involved in stress and protein metabolism (Horst et al.,2005). In addition these cellulytic fungi are reported to have plant growth stimulating effects by enhancing the availability of nutrients and minerals (Fe, N, P) for plants, producing plant growth hormones such as alamenthecins, gliotoxin, harzianic acid, trichotoxin, trichoviridin, viridin, viridiol, etc, and decomposing organic material to improve the soil fertility which produced positive impact on farming production (Kaewchai et al., 2009). *Trichoderma* isolates are reported to improve the nitrogen and phosphorus contents of crops like tomato seedling, sugarcane, etc by enhancing the nitrogen uptake and phosphate solubilization (Azarmi et al., 2011).The same significant improving effect of *T.hamatum* on mineral content especially on percent nitrogen of both non-legume plants was also observed in our study. In addition *Trichoderma* species helped plants to withstand against a biotic stresses such as by increasing the length of secondary roots deep in the ground or soil and improving the water holding capacity to provide protection against drought (Mastouri et al.,2010).Therefore, this stress tolerant disease-free environment and improvement in soil fertility provided by *T.hamatum* may be found effective for rhizobial isolates to promote growth and improving the nutritional status of both non-legume plants asymbiotically or through associative nitrogen fixation in the present study.

Similarly many researchers proved that *rhizobium* and *bradyrhizobium* species are quite competent in surviving and colonizing the rhizospheres of non-legume crops (Jarak et al., 2012; Saharan & Nehra,2011). Studies showed the presence and duplication of *R. legeminosarum bv. trifolii* (strain R39) in rhizosphere of many non-legume crops including barley, corn, radish, rape and wheat (Wiehe & Höflich, 1995), the saprophytic and endophytic presence of *R. etli* in maize roots (Gutiérrez-Zamora & Martínez-Romero,2001) and *bradyrhizobium* species in rice roots **(**Chaintreuil et al.,2000; Chaintreuil et al.,2001). It was also reported that rhizobial inoculation improved the seed germination, seedling emergence and growth of lowland rice variety MR219, another non-legume plant (Mia & Shamsuddin, 2012). Other studies provided evidences that *rhizobium* species can induce not only

increase in germination and seedling emergence but also improved the growth and output of many cereal and non-cereal plants (Mia & Shamsuddin , 2010; Saharan & Nehra,2011). Quite a lot of studies have been reported that *rhizobium* and *bradyrhizobium* species have prominent plant growth improving effects on non-legume plants by several direct and indirect mechanisms. Direct mechanisms include 1. production of phytohormones such as auxin, indole acetic acid, gibberllins, etc, (Humphry et al.,2007; Martínez-Viveros,2010), 2. Increased nutrients uptake (Biswas et al., 2000; Biari et al., 2008), 3. synthesized siderophores which chelate iron (Robin et al.,2008; Avis et al.,2008), 4. increased phosphate solubilization to make phosphate available for plants (Richardson et al., 2009; Yazdani et al., 2009), 5. improved root respiration of inoculated plants (Volpin, & Phillips,1998),6. induced enzyme generation in inoculated plants **(**Ahemad, & Khan, 2011). Whereas the same two genera of nitrogen fixing bacteria also promotes plant growth indirectly by acting as biocontrol agents for plant pathogenic microorganisms through 1. antibiosis by secreting extra-cellular metabolites (antibiotics) against plant pathogenic bacteria and fungi, 2. by producing siderophores to make pathogen starving, 3. by producing hydrogen cyanide (HCN) and 4. by inducting systemic resistance (García-Fraile et al.,2012).

Conclusion:

The obtained results of present study clearly concluded that *T.hamatum*, *rhizobium* and *bradyrhizobium* species alone and in combination are beneficial for the growth and nutritional status of non-legume plants, thereby improving their productivity and most important, they showed synergism by not interfering the natural abilities of one another.

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