



Light Intensity and Biofertilizers Effect on Natural Indigo Production and Nutrient Uptake of *Indigofera tinctoria* L.

D. Setyaningrum¹, M.T.S. Budiastuti², B. Pujiasmanto², D. Purnomo², Supriyono²

10.18805/IJArE.A-507

ABSTRACT

This research investigated the effect of light intensity and biofertilizer on the yield, which includes the production of indigo compounds and plant nutrient uptake. The study used a randomized complete block design with a split plot design with 4 levels of light intensity as the main plots and 4 levels of biofertilizer as a sub plots with 3 replications. The combination of light intensity and biofertilizer affects fresh weight, biomass and tissue nitrogen. The highest fresh weight and biomass was found at 100% light intensity with double inoculation of mycorrhizae and rhizobium. Whereas the highest tissue nitrogen was at 10% light intensity with double inoculation of mycorrhizae and rhizobium. The production of indigo affected by light intensity, ie at 10% light intensity indicates the highest indigo. Mycorrhizae and rhizobium have a synergistic relationship as biofertilizer in increasing plant yields and nutrient uptakes in 100% light intensity.

Key words: Indigo, Mycorrhizae, Nitrogen uptake, Phosphate uptake, Rhizobium.

INTRODUCTION

Synthetic dyes are widely used in the textile industry because they are brighter, cheaper to produce and easily applied to fabric. These dyes are the main group of chemicals that are toxic, carcinogenic and waste can pollute the environment (Attia *et al.* 2008). The waste treatment process is not efficient enough, so the waste is considered a pollutant which is a serious problem in the environment. Therefore, an effort conducted to solve this problem is substituting the synthetic dyes with natural dyes. One of them is *Indigofera tinctoria* L.

Indigofera tinctoria L. is a leguminosae family that can be used as a natural source of indigo dye which was the main product of India and West Africa before the discovery of synthetic tilapia. The plant is used as a natural coloring agent because it contains indigo pigments which produce indigo colors (Hariri *et al.* 2017). Natural coloring compounds extracted from plant leaves that contain indican secondary metabolites (indoxyl- β -D-glucoside) as a precursor of indigo that are produced in leaf vacuoles (Inouea *et al.* 2017). Indoxyl- β -D-glucoside hydrolyzed to indoxyl and oxidized air to indigo (Fig 1). Indigo compound production is very responsive to sunlight (Stoker *et al.* 1998). Indigo is a precursor compound metabolites that contain nitrogen and are produced through the shikimic acid pathway. Nitrogen-containing metabolites (alkaloids, glucosinolates and cyanogen glycosides) increase with reduced light (Coelho *et al.* 2007), while growth and plant biomass are optimal at high light intensities (Wu *et al.* 2017). So that nutrition is needed to balance the growth and production of indigo.

One effort to fulfill nutrition with biofertilizer application is mycorrhizae and rhizobium. Colonization of the roots of legume plants with mycorrhizae and rhizobium has a positive effect on plant growth (Sprent 2001; van der Heijden 2006), including increased vegetative growth and yield (Sefapour *et al.* 2011). In legume trees, both microbes can have double

¹Graduate School of Agronomy, Universitas Sebelas Maret, Indonesia.

²Departement of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret, Indonesia.

Corresponding Author: D. Setyaningrum, Graduate School of Agronomy, Universitas Sebelas Maret, Indonesia.

Email: desysetyaningrum23@student.uns.ac.id

How to cite this article: Setyaningrum, D., Budiastuti, M.T.S., Pujiasmanto, B., Purnomo, D. and Supriyono (2020). Light Intensity and Biofertilizers Effect on Natural Indigo Production and Nutrient Uptake of *Indigofera tinctoria* L. Indian Journal of Agricultural Research. 54(5): 578-584.

Submitted: 18-11-2019 **Accepted:** 06-04-2020 **Published:** 18-06-2020

symbiosis (Franzini *et al.* 2010). However, most of the research that has been done is in optimal conditions. While research on mycorrhizal and rhizobium colonization of legume plants at some light intensities is very limited. In several previous studies that there was an interaction between mycorrhizal inoculation and rhizobium with light intensity on Lima Bean (*Phaseolus lunatus* L.) (Ballhorn *et al.* 2016). Application of a combination of organic fertilizer and biofertilizer can increase indican production, herbal yields of *Indigofera tinctoria* plants and the content of N and K in the soil (Shindu *et al.* 2016). Mycorrhizae inoculation affects growth and phytochemicals of *I. tinctoria* (Sundar *et al.* 2012). The novelty of this research is to use *Indigofera tinctoria* as a host plant to determine the symbiotic relationship of mycorrhizae and rhizobium in several light intensities on yield, indigo production and nutrient uptake.

MATERIALS AND METHODS

The study was conducted from April to August 2019 in Sukoharjo, Central Java, Indonesia. The research site location was 110° 51' 49.44" East Longitude and 7° 48' 54.3"

South latitude with an altitude of 120 meters above sea level. Microclimatic conditions including an average rainfall of 2,289 mm per year within 105 days of rainy day in average. Soil chemical and physical analysis was carried out by taking soil in a composite manner after land management. Based on chemical and physical analysis of the soil that the soil in research field has pH (H₂O) 7.31 (neutral), field capacity 17.4%, cation exchange capacity 56.93 me.100 g⁻¹ (low), C organic 1.52% (low), organic matter 2.62% (low), total nitrogen 0.36% (low), available phosphate 15.72 ppm (low), available potassium 0.42 me.100g⁻¹ (low) and structured soil was loam slob with clay content 28.67, silt 27.91 and sand 43.40. The content criteria were based on Soil Research Institute (2009). Environmental conditions were observed starting 1 week after planting - 12 weeks after planting. Observation of light intensity was carried out inside the canopy using luxmeter. The observation results of research site's environmental conditions were shown as follows (Table 1).

The research used a Randomized Complete Block Design (RCBD) arranged with a split plot. The level of light intensity as the main plots consisted of N1 = 100% (full light), N2 = 50% (50% of incoming light); N3 = 25% (25% incoming light); N4 = 10% (10% incoming light). The application of biofertilizer as sub plots consisted of B1 = without biofertilizer, B2 = rhizobium 1 g.plant⁻¹; B3 = mycorrhizae 10 g.plant⁻¹; B4 = 10 mycorrhizae and rhizobium 1 g.plant⁻¹. Each experiment unit was repeated 3 times to obtain 48 experimental units. *Rhizobium* sp was applied to seeds during nursery. The nursery was carried out for up to 4 weeks, then the plants are transplanting to the research field. Mycorrhizal treatment was carried out when transplanting plants in the research field. The instrument used as an application of light intensity was parantet with various density levels.

Analysis of nitrogen and phosphate uptake referred to the book Soil Research Institute (2009). Analysis of N application was done by Kjeldahl method and P analysis using a wet ashing method with HNO₃ and HClO₄. Leaf

samples were harvested in the early generative phase which then dried in the oven for 2 days at 60°C and grounded to powder. Observation of biomass was done by drying the sample of the plant with an oven temperature of 60°C for 48 hours. After drying, the weight of the plant sample was recorded as plant biomass. Nutrient uptake was calculated by multiplying the dry matter yield with nutrient content and expressed in g.plant⁻¹.

Nutrient uptake (g.plant⁻¹) =

$$\text{Nutrient content (\% in dry matter)} \times \frac{\text{yield of dry matter (g.plant}^{-1}\text{)}}{100}$$

Indigo dye extraction done by fermenting leaves cut in water at 48 hours, added with Ca(OH)₂ in proportion of 2.5% of leaves weight and centrifuged at 9820xg for 10 minutes (Chanayath *et al.* 2002). Indigo compounds were analyzed using spectrophotometer (Wu *et al.* 1999). Indigo concentration was calculated using indigo calibration curves.

Table 1: The average value of environmental conditions in the research site.

Light Intensity Treatment	Environmental Variable	Observation Time		
		07.00 am	12.00 am	05.00 pm
100%	Light Intensity (cd.m ⁻²)	3203.85	6232.8	1915.95
	Air Temperature (°C)	28	34.6	29.8
	Relative Humidity (%)	61	43	54
50 %	Light Intensity (cd.m ⁻²)	1613.85	3013.05	957.97
	Air Temperature (°C)	27,2	33.1	29.0
	Relative Humidity (%)	63	50	56
25%	Light Intensity (cd.m ⁻²)	898.35	1605.9	478.98
	Air Temperature (°C)	26.6	32.8	28.7
	Relative Humidity (%)	66	51	57
10%	Light Intensity (cd.m ⁻²)	300.51	623.99	190.64
	Air Temperature (°C)	29.8	31.2	28.2
	Relative Humidity (%)	68	52	60

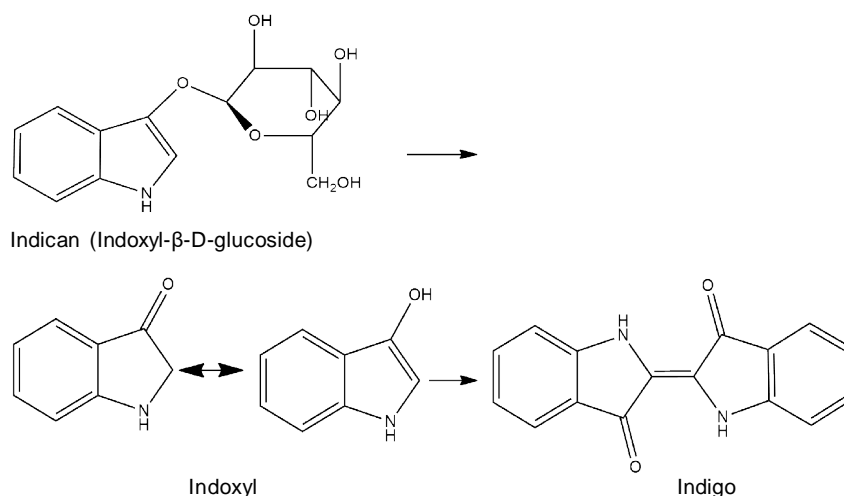


Fig 1: Indigo production pathway in *Indigofera tinctoria* (Chanayath *et al.* 2002).

The calibration curve was carried out using various standard amounts of indigo, obtained by dissolving 8 mg of standard indigo in 20 ml of H₂SO₄ and diluted to 500 ml with distilled water. The solution was then diluted at different concentrations with a solution of H₂SO₄ (H₂SO₄: distilled water; 1:24) and absorbance measured at 611 nm. Indigo paste samples were dissolved in 20 ml H₂SO₄, diluted to 500 ml with distilled water and the absorbance was measured at 611 nm. Indigo content of the sample was calculated from the polynomial distribution curve derived from the absorbance of a series dilution of a standard indigo solution.

Observation variables included fresh plant weight, biomass and indigo analysis carried out when the plant was 12 weeks after planting. Tissue nitrogen, nitrogen uptake and phosphate uptake were carried out at 10 weeks after planting. Research data were analyzed using analysis of variance with a 5% test level (confidence level 95%). Further analysis of Duncan's Multiple Range Test (DMRT) was done upon significant difference within the variable observed.

RESULTS AND DISCUSSION

Fresh weight

Light intensity affected the fresh weight of plants (Table 2). The highest fresh weight was at 100% light intensity and decreased with decreasing light intensity. Fresh weight at 50% light intensity decreased 32% and at light intensity 25% and 10% decreased 54% and 86% compared to 100% light intensity. Tiwari *et al.* (2015) that high plant light interception caused an increase in the number of branches and tillers so that the crop yield would be high. This is because the main response of plants during photosynthesis is entirely dependent on light conditions. The application of biofertilizer also affected fresh weight (Table 2). Fresh weight with

rhizobium inoculation increased 45% compared to without biofertilizer. This is because *Indigofera tinctoria* is a type of legume that can be symbiotic with rhizobium. In *cluster bean*, rhizobium inoculation can increase the number of leaves and fresh weight (Gul *et al.* 2019). Double inoculation of mycorrhizae and rhizobium increased fresh weight by 57%.

The interaction between light intensity and biofertilizer significantly affected fresh weight (Table 2). The highest fresh weight (782.65 g) found at a combination of 100% light intensity and double inoculation of mycorrhizae and rhizobium. While the lowest yield (39.98 g) in the combination of 10% light intensity and without biofertilizer. Rhizobium and mycorrhizae have a synergistic relationship as biofertilizer in promoting growth, nodulation and nitrogen fixation in soybeans (Younesi *et al.* 2013). However, this effect tends to be higher under full light, whereas in low light conditions indicate competition for photosynthate. The synergistic effect under full light was reported in this and other studies (Mortimer *et al.* 2008).

Biomass

Light intensity affected plant biomass (Table 3). The highest biomass (16.70 g) found at 100% light intensity. Biomass decreased 42% and 62% at light intensities of 25% and 10% compared to 100% light intensity. This is presumably because biomass is a product of photosynthesis which depends on the availability of light to produce carbohydrates. Low light intensity causes the level of net photosynthesis to decrease so that plant biomass is also reduced (Su *et al.* 2014). Biofertilizers also have a significant effects on biomass (Table 3). The highest biomass (15.92 g) found in double inoculation of mycorrhizae and rhizobium. Besides that, the interaction of light intensity and biofertilizer affected biomass (Table 3). The combination of 100% light intensity with mycorrhizae and rhizobium showed the highest biomass

Table 2: Effect of light intensity and biofertilizer on the fresh weight (g).

Light Intensity	Biofertilizer				Average
	Without biofertilizer	Mycorrhizae	Rhizobium	Mycorrhizae + Rhizobium	
100%	360.37 ^{cd}	402.67 ^{de}	625.65 ^{ef}	782.65 ^f	542.83 ^d
50%	382.36 ^{cde}	320.25 ^{bcd}	425.39 ^{de}	347.69 ^{bcd}	368.92 ^c
25%	184.58 ^{abcd}	251.45 ^{abcd}	262.18 ^{abcd}	281.84 ^{abcd}	245.01 ^b
10%	39.98 ^a	50.70 ^a	91.50 ^{ab}	115.58 ^{abc}	74.44 ^a
Average	241.82 ^a	256.27 ^{ab}	351.18 ^{bc}	381.94 ^c	

Description: The figure followed by the same letters show no significant differences based on DMRT level of 5%.

Table 3: Effect of light intensity and biofertilizer on the biomass (g).

Light Intensity	Biofertilizer				Average
	Without biofertilizer	Mycorrhizae	Rhizobium	Mycorrhizae + Rhizobium	
100%	6.17 ^{abc}	13.57 ^{cde}	22.09 ^{ef}	24.97 ^f	16.70 ^b
50%	13.67 ^{cde}	10.44 ^{abcd}	14.70 ^{cde}	19.41 ^{def}	14.57 ^b
25%	3.55 ^{ab}	10.94 ^{abcd}	13.38 ^{bode}	10.57 ^{abcd}	9.61 ^a
10%	2.78 ^a	7.36 ^{abc}	6.37 ^{abc}	8.71 ^{abc}	6.30 ^a
Average	6.54 ^a	10.58 ^b	14.15 ^{bc}	15.92 ^c	

Description: The figure followed by the same letters show no significant differences based on DMRT level of 5%.

Table 4: Effect of light intensity and biofertilizer on tissue nitrogen (%).

Light Intensity	Biofertilizer				Average
	Without biofertilizer	Mycorrhizae	Rhizobium	Mycorrhizae + Rhizobium	
100%	6.68 ^a	15.20 ^b	16.80 ^{bc}	17.40 ^{bc}	14.02 ^a
50%	15.90 ^b	20.50 ^{cde}	22.10 ^e	22.00 ^e	20.12 ^b
25%	18.00 ^{bcd}	22.70 ^e	23.40 ^{ef}	24.50 ^{ef}	22.18 ^c
10%	21.30 ^{de}	23.50 ^{ef}	22.30 ^e	26.80 ^f	23.51 ^c
Average	15.49 ^a	20.49 ^b	21.16 ^{bc}	22.69 ^c	

Description: The figure followed by the same letters show no significant differences based on DMRT level of 5%.

Table 5: Effect of light intensity and biofertilizer on indigo production (mg/l).

Light Intensity	Biofertilizer				Average
	Without biofertilizer	Mycorrhizae	Rhizobium	Mycorrhizae + Rhizobium	
100%	0.56	0.85	0.51	3.92	1.46 ^a
50%	3.79	3.29	4.27	4.98	4.08 ^b
25%	5.89	4.65	5.21	4.22	4.99 ^b
10%	5.72	5.37	4.21	7.19	5.62 ^b
Average	3.99 ^a	3.542 ^a	3.55 ^a	5.08 ^a	

Description: The figure followed by the same letters show no significant differences based on DMRT level of 5%.

with 24.97 g. Muhamed *et al.* (2019) that an increase in biomass with the application of mycorrhizae is associated with a gradual increase in nodulation, nitrogen fixation and nutrition. Mycorrhizae also exhibit plant growth promoters, including the production of indole acetic acid (IAA) (Richardson *et al.* 2009). Mycorrhizae and rhizobium inoculations were able to increase *Pisum sativum* L. biomass by 43% in full light compared to low light. This is because the reduced light intensity significantly suppresses mycorrhizal colonization and symbiosis of rhizobium (Reinhard *et al.* 1994).

Tissue nitrogen

Light intensity affected tissue nitrogen (Table 4). The highest tissue nitrogen (23.51%) found at light intensity of 10% and decreases with increasing light received by plants. This is allegedly due to the high light intensity which can accelerate the occurrence of evaporation and leaching of nutrients especially mobile nutrients. The results of the study by Li *et al.* (2011) that the reduced light received by plants can maximize nitrogen fixation. The results also showed that biofertilizer was able to significantly increase tissue nitrogen. The lowest tissue nitrogen (15.49%) found at the treatment without biofertilizer. Mycorrhizae inoculation was able to increase tissue nitrogen by 24% compared to without biofertilizer. Rhizobium inoculation increased tissue nitrogen by 26% compared to without biofertilizer. The interaction between light intensity and biofertilizer significantly affected tissue nitrogen (Table 4). The highest tissue nitrogen (26.8%) found in the combination of 10% light intensity with double inoculation of mycorrhizae and rhizobium. The association of mycorrhizae and rhizobium directly improves the nutrition status and growth of legume (Hao *et al.* 2019). In addition, the reduced light received by plants can maximize nitrogen fixation (Li *et al.* 2011). Legumes are associated with

arbuscular mycorrhizal fungi and rhizobium, thus increasing nutrition, namely P and N for host plants (Larimer *et al.* 2014).

Indigo production

The results showed that light intensity had an effect on the concentration of indigo concentration (Table 5). These results are in accordance with Stoker *et al.* (1998) that light intensity affects indigo production in *Isatis tinctoria*. The highest indigo concentration (5.62 mg/L) found at 10% intensity and indigo decreased with increasing light intensity. Indigo decreases 74% in full light compared to 10% light intensity. These results indicated that low light stimulates biosynthesis or accumulation of secondary metabolites. In fact, low light intensity is considered an environmental stimulus in the production of secondary metabolites. However, the concentration of indigo per unit weight of *Polygonum tinctorium* leaf increases with exposure to higher light intensities before harvest (Campeol *et al.* 2006). Indigo Precursor is a metabolite compound that contains nitrogen. In the shikimic acid pathway, precursors derived from glycolysis and pentose phosphate converted to aromatic amino acids (Taiz and Zeiger 2006). Nitrogen-containing metabolites (alkaloids, glucosinolates and cyanogen glycosides) increase with reduced light (Coelho *et al.* 2007).

The results of this study indicated that there was no effects of biofertilizer and no interaction between light intensity and biofertilizer on indigo production. Based on research by Sindhu *et al.* (2016) that the application of

Table 6: Correlation of indigo production and nitrogen tissue.

	Light intensity	Indigo	Nitrogen tissue
Light intensity	1	0.576**	0.679**
Indigo	0.576 **	1	0.512**
Nitrogen tissue	0.679**	0.512**	1

**Correlation is significant at the 0.01 level (2-tailed).

Table 7: Effect of light intensity and biofertilizer to nitrogen uptake (g.plant⁻¹).

Light Intensity	Biofertilizer				Average
	Without biofertilizer	Mycorrhizae	Rhizobium	Mycorrhizae + Rhizobium	
100%	48.11	215.13	318.51	340.12	230.47 ^b
50%	123.25	215.34	296.24	310.31	236.29 ^b
25%	59.69	246.79	286.64	260.33	213.37 ^b
10%	59.48	174.26	144.75	234.47	153.22 ^a
Average	72.64 ^a	212.86 ^b	261.54 ^{bc}	286.31 ^c	

Description: The figure followed by the same letters show no significant differences based on DMRT level of 5%.

Table 8: Effect of light intensity and biofertilizer to phosphate uptake (g.plant⁻¹).

Light Intensity	Biofertilizer				Average
	Without biofertilizer	Mycorrhizae	Rhizobium	Mycorrhizae + Rhizobium	
100%	3.13	9.11	9.71	12.11	8.52 ^b
50%	4.15	7.19	8.83	9.83	7.50 ^b
25%	1.64	6.15	6.63	6.47	5.22 ^a
10%	1.39	4.13	3.52	7.21	4.06 ^a
Average	2.58 ^a	6.64 ^b	7.17 ^{bc}	8.90 ^c	

Description: The figure followed by the same letters show no significant differences based on DMRT level of 5%.

biological fertilizer and mycorrhizae can increase indigo precursors. Based on Table 6 that indigo concentration is positively correlated with tissue nitrogen. Nitrogen is one of the constituent elements of indigo. Nitrogen is the main element of various organic compounds such as amino acids, proteins, nucleic acids and secondary metabolic compounds such as alkaloids (Mengel *et al.* 2001). Indigo is a synthesis of indoxyl- β -D-glucoside precursor molecules derived from plant secondary metabolites. These precursors are thought to originate the same as indole, ie, from the shikimic acid pathway, either through tryptophan or indole-3-pyruvate (Xia and Zenk 1992).

Nitrogen uptake

Light intensity affected nitrogen uptake (Table 7). The highest nitrogen uptake (236.29 g.plant⁻¹) found at 100% light intensity and not significantly different from 50% and 25% light intensity. This is because light intensity significantly affect growth, nutrient uptake and efficiency ratio of nutrient use (Baligar *et al.* 2006). Increased nitrogen uptake at 100% light intensity can be attributed to higher plant biomass at 100% light intensity. There is a relationship between the accumulation of plant nutrients with biomass production (Rasmussen and Gengenbach 1994). Light intensity affect the expectation of NO₃ (Lee *et al.* 2017).

Table 7 showed that biofertilizer also affected nitrogen uptake. The highest nitrogen uptake (286.31 g.plant⁻¹) found in double inoculation of mycorrhizae and rhizobium. Some research also shows that mycorrhizae and rhizobium inoculation can promote plant growth and increase crop yields and crop nutrient uptake (Abd-Alla *et al.* 2014). This is presumably due to the symbiosis of legume-rhizobium which plays a role in nitrogen fixation through nodulation in legume roots (Singh and Singh 2018) and rhizobium have a synergistic effect with mycorrhizae (Sharma *et al.* 2012).

Shindu *et al.* (2016) found that the uptake of Nitrogen and Phosphate *Indigofera tinctoria* reached 365.37 and 18.45 kg.ha⁻¹ with mycorrhizal application, manure and azospirillum. The combination of light intensity and biofertilizer did not significantly affects nitrogen uptake. Rhizobium and mycorrhizae inoculation can increase nitrogen fixation in low light so that nitrogen uptake also increases (Meng *et al.* 2015).

Phosphate uptake

Light intensity significantly affected phosphate uptake (Table 8). Highest phosphate uptake (8.52 g.plant⁻¹) found at 100% light intensity. Phosphate uptake at 100% light intensity was not significantly different from 50% light intensity. The results of this study are in line with Zhou *et al.* (2019) that the highest phosphate uptake and dry weight of plants found at high light intensity. Biofertilizer also had a significant effect on phosphate uptake (Table 8). The highest uptake of phosphate (8.90 g.plant⁻¹) found in double inoculation of mycorrhizae and rhizobium. This is presumably because the roots of *Indigofera tinctoria* form a mutualistic symbiotic with soil microorganisms such as mycorrhizae and rhizobium. The synergistic effect between mycorrhizae and rhizobium with legume root causes an increase in nutrient uptake (Li *et al.* 2012). Mycorrhizal fungi increases nutrient uptake especially phosphate (Meng *et al.* 2015) because mycelium can grow and expand outside the rhizosphere, connecting roots with soil micro-habitat and enlarging the root area so that it absorbs more nutrients. Thus, water and nutrients can be transported by large hyphal tissue to be absorbed by plants (Liu *et al.* 2010).

CONCLUSION

The highest fresh weight and biomass found at 100% light intensity with double inoculation of mycorrhizae and

rhizobium. Whereas the highest tissue nitrogen found in the combination of 10% light intensity with double inoculation of mycorrhizae and rhizobium. The production of indigo was affected by light intensity, *i.e* at 10% light intensity indicated the highest indigo concentration. Indigo concentration was positively correlated with tissue nitrogen. Therefore, Mycorrhizae and rhizobium have a synergistic relationship as biofertilizer in increasing plant yields and nutrient uptakes in 100% light intensity.

REFERENCES

- Abd-Alla, M.H., El-Enany, A.E., Nafady, N.A., Khalaf, D.M., Morsy, F.M. (2014). Synergistic interaction of Rhizobium leguminosarum bv.viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiological Research. 169: 49-58. DOI: 10.1016/j.micres.2013.07.007.
- Attia, A.A., Girgis, B.S., Fathy, N.A. (2008). Removal of methylene blue by carbons derived from peach stones by H₃PO₄ activation: Batch and column studies. Dyes and Pigments. 76: 282-289.
- Baligar, V.C., Fageria, A.Q., Paiva, A., Silveira, A.W.V., Pomella, R.C.R., Machado. (2006). Light intensity effects on growth and micronutrient uptake by tropical legume cover crops. Journal of Plant Nutrition. 29: 1959-1974.
- Ballhorn, D.J., Schädler, M., Elias, J.D., Millar, J.A., Kautz, S. (2016). Friend or Foe-Light Availability Determines the Relationship between Mycorrhizal Fungi, Rhizobia and Lima Bean (*Phaseolus lunatus* L.). PLoS ONE. 11: 1-12. DOI:10.1371/journal.pone.0154116.
- Campeol, E., Luciana, G., Angelini, Tozzi, S., Bertolacci M. (2006). Seasonal variation of indigo precursors in *Isatis tinctoria* L. and *Polygonum tinctorium* Ait. as affected by water deficit. Environmental and Experimental Botany. 58: 223-233.
- Chanayath, N., Lhieochaiphant, S., Phutrakul, S. (2002). Pigment Extraction Techniques from the Leaves of *Indigofera tinctoria* Linn. and *Baphicacanthus cusia* Brem. and Chemical Structure Analysis of Their Major Components. Chiang Mai University Journal. 1: 149.
- Coelho, G.C., Rachwal, M.F.G., Dedecek, R.A., Curcio, G.R., Nietsche, K., Schenkel, E.P. (2007). Effect of light intensity on methylxanthine contents of *Ilex paraguariensis* A. St. Hil. Biochemical Systematics and Ecology. 35: 75-80.
- Franzini, V.I., Azcón, R., Mendes, F.L., Aroca, R. (2010). Interactions between Glomus species and Rhizobium strains affect the nutritional physiology of drought-stressed legume hosts. J. Plant Physiol. 167: 614-619. DOI: 10.1016/j.jplph.2009.11.010 PMID: 20044167.
- Gul, A., Salam, A., Afridi, M.S., Bangash, N.K., Ali, F., Ali, M.Y., Khan, S., Mubeen, R. (2019). Effect of urea, bio-fertilizers and their interaction on the growth, yield and yield attributes of *Cyamopsis Tetragonoloba*. Indian Journal of Agricultural Research. 53: 423-428.
- Hao, Z., Xie, W., Jiang, X., Wu, Z., Zhang, X., Chen, B. (2019). Arbuscular Mycorrhizal Fungus Improves Rhizobium-*Glycyrrhiza* Seedling Symbiosis under Drought Stress. Agronomy. 9: 572.
- Hariri, M.F., Chikmawati, T., Hartana, A. (2017). Genetic diversity of *Indigofera tinctoria* L. in Java and Madura islands as natural batik dye based on intersimple sequence repeat markers. J. Math Found Sci. 49: 105-115.
- Inouea, S., Moriayaa, T., Moritaa, R., Kuwatab, K., Thulc, S.T., Bijaya, K., Sarangic., Minamia, K. (2017). Characterization of UDP-glucosyltransferase from *Indigofera tinctoria*. Plant Physiology and Biochemistry. 121: 226-233.
- Larimer, A.L., Clay, K., Bever, J.D. (2014). Synergism and context dependency of interactions between arbuscular mycorrhizal fungi and rhizobia with a prairie legume. Ecology. 95: 1045-1054.
- Lee, K.H., Jeong, H.J., Kim, H.J., Lim, A.S. (2017). Nitrate uptake of the red tide dinoflagellate *Prorocentrum micans* measured using a nutrient repletion method: effect of light intensity. Algae. 32: 139-153. DOI.org/10.4490/algae.2017.32.5.20.
- Li, A.R., Smith, F.A., Smith, S.E., Guan, K.Y. (2012). Two sympatric root hemiparasitic Pedicularis species differ in host dependency and selectivity under phosphorus limitation. Functional Plant Biology. 39: 784-794.
- Li, Q.Z., Sun, J.H., Wei, X.J., Christie, P., Zhang, F.-S., Li, L. (2011). Overyielding and interspecific interactions mediated by nitrogen fertilization in strip intercropping of maize with faba bean, wheat and barley. Plant and Soil. 339: 147-161.
- Liu, B., Liu, X, B., Liu, C., Wang, Y.S., Li, J., Herbert, S.J. (2010). Soybean yield and yield component distribution across the main axis in response to light enrichment and shading under different densities. Plant Soil Environment. 56: 384-392.
- Meng, L., Zhang, A., Wang, F., Han, X., Wang, D., Li, S. (2015). Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Frontiers in Plant Science. 6: 339. DOI: 10.3389/fpls.2015.00339.
- Mengel, K., E. A. Kirkby, H. Kosegarten., T. Appel. (2001). Principles of Plant Nutrition, 5th edition. Kluwer Academic Publishers, Dordrecht, Netherlands.
- Mohamed, I., Eidd, K.E., Mohamed, H., H. Abbasb, Ahmed, A., Saleme., Ahmedf, N., Alib, M., Shahg, G.M., Fanga, C. (2019). Use of plant growth promoting Rhizobacteria (PGPR) and mycorrhizae to improve the growth and nutrient utilization of common bean in a soil infected with white rot fungi. Ecotoxicology and Environmental Safety. 171: 539-548.
- Mortimer, P.E., Perez-Fernandez, M.A., Valentin, A.J. (2008). The role of arbuscular mycorrhizal colonization in the carbon and nutrient economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. Soil Biology and Biochemistry. 40: 1019-1027.
- Reinhard, S., Weber, E., Martin, P., Marschner, H. (1994) Influence of phosphorus supply and light intensity on mycorrhizal response in *Pisum-rhizobium-glomus* symbiosis. Experientia. 50: 890-896.
- Richardson, A.E., Barea, J.M., McNeill, A.M., Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by micro-organisms. Plant and Soil. 321: 305-339. doi:10.1007/s11104-009-9895-2.

- Sefapour, M., Ardakani, M., Khaghani, S., Rejali, F., Zargari, K., Changiz, M., *et al.* (2011). Response of yield and yield components of three red bean (*Phaseolus vulgaris* L.) genotypes to co-inoculation with glomus intraradices and *Rhizobium phaseoli*. *Am. J. Agric. Environ. Sci.* 11: 398-405.
- Sharma, M.P., Jaisinghani, K., Sharma, S.K. and Bhatia, V.S. (2012). Effect of native soybean rhizobia and AM fungi in the improvement of nodulation, growth, soil enzymes and physiological status of soybean under microcosm conditions. *Agricultural Research.* 1: 346-351. doi:10.1007/s40003-012-0038-2.
- Sindhu, P.V., Kanakamany, M.T., Beena, C. (2016). Effect of organic manures and biofertilisers on herbage yield, quality and soil nutrient balance in *Indigofera tinctoria* cultivation. *Journal of Tropical Agriculture.* 54: 16-20.
- Singh, Z and Singh, G. (2018). Role of rhizobium in chickpea (*Cicer arietinum*) production - A review. *Agricultural Reviews.* 39: 31-39.
- Soil research center. (2009). *Chemical Analysis of Soil, Plants, Water and Fertilizer.* Soil Research Institute, Bogor.
- Sprent, J.I. (2001). *Nodulation in Legumes.* Royal Botanic Gardens, Kew, UK.
- Stoker, K.G., David, T., Cooke., David, J., Hill. (1998). Influence of light on natural indigo production from woad (*Isatis tinctoria*). *Plant Growth Regulation.* 25: 181-185.
- Su, B.Y., Song, Y.X., Song, C., Cui, L., Yong, T.W. and Yang, W.Y. (2014). Growth and photosynthetic responses of soybean seedlings to maize shading in relay intercropping system in Southwest China. *Photosynthetica.* 52: 332-340. doi: 10.1007/s11099-014-0036-7
- Sundar, S.K., Palavesam, A., Parthipan, B. (2012). Studies on the synergistic effect of AM fungi and PGPRS on growth and phytochemical properties of medicinally important *Indigofera tinctoria* L. *Journal of Pharmacy Research.* 5: 3990-3993.
- Taiz, L. and Zeiger, E. (2006). *Plant Physiology.* 4th Edition, Sinauer Associates Inc. Publishers Massachusetts.
- Tiwari, R., Yadav, R.S., Kumawat, A. (2015). Evaluation of pearl millet (*Pennisetum glaucum*) and clusterbean (*Cyamopsis tetragonoloba* L.) intercropping system under arid western plain zone in India. *Indian Journal of Agricultural Research.* 49: 229-234.
- Van der Heijden, M.G.A., Streitwolf-Engel, R., Riedl, R., Siegrist, S., Neudecker, A., Ineichen, K., *et al.*, (2006). The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist.* 172: 739-752.
- Wu, E., Komolpis, K., Wang, H.Y. (1999). Chemical extraction of indigo from *Indigofera tinctoria* while attaining biological integrity. *Biotechnology Techniques.* 13: 567-569.
- Wu, Y.S., Yang, F., Gong, W.Z., Ahmed, S., Fan, Y.F., Wu, X.L., Yong, T.W., Liu, W.G., Shu, K., Liu, J., Du, J.B., Yang, W.Y. (2017). Shade adaptive response and yield analysis of different soybean genotypes in relay intercropping systems. *J. Integr. Agric.* 16(6): 1331-1340.
- Xia, Z., Zenk, M. (1992) Biosynthesis of indigo precursors in higher plants. *Photochemistry.* 31: 2695-2697.
- Younesi, O., Moradi, A., Namdari, A. (2013). Influence of arbuscular mycorrhiza on osmotic adjustment compounds and antioxidant enzyme activity in nodules of salt-stressed soybean (*Glycine max*). *Acta Agriculturae Slovenica.* 101: 219-230.
- Zhou T., Wang, L., Li, S., Gao, Y., Du, Y., Zhao, L., Liu, W., Yang, W. (2019) Interactions between light intensity and phosphorus nutrition affect the p uptake capacity of maize and soybean seedling in a low light intensity area. *Front. Plant Sci.* 10:183. doi: 10.3389/fpls.2019.00183.