Effects of farmyard manure, NPK Levels, and foliar nutrition on chemical and biochemical parameters of true indigo (*Indigoferatinctoria* L.)

ABSTRACT

The use of plants as natural dyes in the textile industry has gained significant attention in recent years. *Indigoferatinctoria* L., a member of the Fabaceae family native to India, has emerged as a promising sustainable dye source. Beyond its applications in textiles, *I. tinctoria* also holds significance in herbal medicine, highlighting its multifaceted utility.A two-year field experiment (2022 and 2023) in split split plot design was conducted at the College of Agriculture, Vellanikkara, Kerala Agricultural University, to investigate the effects of farmyard manure (FYM) [5, 7.5, and 10 t ha^{-1}], NPK levels [10:10:20, 20:10:40, 30:10:60, and 40:10:80 kg ha^{-1}], and foliar nutrition [water spray, nano urea 0.4%, and seaweed extract 0.4%] on the indican content and nutrient content of indigo plants. The results showed that FYM [10 t ha^{-1}] increased the indican content [by 8.73%], nitrogen [by 11.02%], phosphorus [by 9.9%], and potassium [by 11.27 %]. NPK levels [30:10:60 kg ha^{-1} and 40:10:80 kg ha^{-1}] showed similar effects on indican and nutrient content. Foliar application of seaweed extract [0.4%] increased indican and phosphorus content. The interaction between FYM, NPK levels, and foliar nutrition significantly affected the indican, nitrogen, and potassium contents. The study suggests that integrated use of FYM [10 t ha^{-1}], NPK [30:10:60 kg ha^{-1}], and foliar nutrition with seaweed extract [0.4%] can improve plant chemical and biochemical parameters.

Keywords: True indigo, Indigofera, indican, seaweed, nano urea, FYM, Biostimulants

1. INTRODUCTION

The world is witnessing a remarkable resurgence of interest in botanical natural products, leading to a significant surge in the development of herbal medicines, phytonutrients, and nutraceuticals. This trend is fueled by a growing recognition of herbal medications' compatibility, lower toxicity, and affordability. According to the World Health Organization (WHO), a staggering 80% of the global population relies on traditional medicine, which is largely based on plant-derived products (WHO, 2019). With its ancient Ayurveda and herbal remedies tradition, India is uniquely positioned to capitalize on this trend.

Among the various medicinal plants, *Indigoferatinctoria* L., the source of true indigo, holds great significance due to its versatile applications in the dyeing and herbal medicine industries. Indigo, derived from *I. tinctoria*, is one of the oldest naturally obtained coloring agents, with a history of use dating back to the 7th century BC (Kriger, 2006).*I. tinctoria*is a medicinal plant traded in high volume (≥100tonnes/year) prioritized by the State Medicinal Plant Board, Kerala.The plant's herbage is highly valued in the market, displaying a distinctive blue hue. Recently, interest in natural dyes, including indigo, has been revived for coloring food, pharmaceutical, cosmetic, and textile products (Ensley *et al.*, 1983).The blue pigment characteristic of indigo is primarily attributed to the presence of glycosides. Indican, a tryptophan-derived compound, is the precursor to indigo and is a colorless, water-soluble molecule (Gaboriaud-Kolar, 2014).

Indican biosynthesis and its conversion to indigo in *I. tinctoria* is influenced by a complex interplay of factors, including plant nutritional status, environmental conditions, and processing methodologies. Therefore, optimizing the yield and quality of true indigo necessitates a thorough understanding of integrated nutrient management strategies, incorporating farmyard manure, NPK levels, and foliar nutrition. This knowledge is crucial for developing sustainable and eco-friendly cultivation practices, ultimately enhancing indigo production and livelihoods. Despite the established benefits of biostimulants in enhancing crop yields and quality, their effects on true indigo's chemical and biochemical parameters remain poorly understood, highlighting the need for further investigation.

This study investigated the effect of FYM, NPK and foliar nutrition on the chemical and biochemical parameters of true indigo (*Indigoferatinctoria* L.), focusing on optimizing the production of indican. The results of this study will contribute to the development of sustainable production practices for true indigo and provide insights into the use of biostimulants in medicinal plant production.

2. MATERIAL AND METHODS

The field experiment was conducted in the Agronomy field of the College of Agriculture, Vellanikkara, Kerala Agricultural University. Geographically, the site is situated at 10.55°N latitude and 76.28°E longitude, with an altitude of 22.5 m above mean sea level. The region experiences a warm, tropical, humid climate. The soil type at the trial location was laterite. The experiment was laid out in a split-split plot design, with farmyard manure (FYM) as the main plot treatment, fertilizer levels as subplot (1) treatments, and foliar nutrition as subplot (2) treatments. Planting was done at a spacing of 45 cm × 30 cm in plots measuring 3.15 m × 3 m. The field experiment was initiated in September 2022, and a confirmatory trial was conducted in 2023.

The experiment consisted of three levels of main plot treatments: M_1 (5 t ha^{-1} FYM), M_2 (7.5 t ha^{-1} FYM), and M_3 (10 t ha^{-1} FYM). The subplot (1) treatments comprised four levels of fertilizer: S_1 (10:10:20 kg NPK ha^{-1}), S_2 (20:10:40 kg NPK ha^{-1}), S_3 (30:10:60 kg NPK ha^{-1}), and S_4 (40:10:80 kg NPK ha^{-1}). The subplot (2) treatments consisted of three levels of foliar nutrition: SS_1 (water spray), SS_2 (0.4% nano urea foliar application), and SS_3 (0.4% seaweed extract (IFFCO Sagarika) foliar application).

2.1 Manures and fertilizers

To maintain optimal soil pH, lime was applied at a rate of 600 kg $CaCO_3^{-1}$ after evaluating the soil acidity post-ploughing. The soil was low in available N (234 kg ha^{-1}), highin available P and high in available K (41.6 kg ha^{-1}). Before planting, farmyard manure was incorporated into the soil as per treatments. Subsequently, the plots received fertilizer applications according to their designated treatment levels, using urea, diammonium phosphate (Raj Phos), and muriate of potash. The full recommended doses of nitrogen and phosphorus were applied as a baseline. At the same time, potassium was split into two applications: half as a basal dose and the remaining half as a follow-up dose at 90DAP and 135 DAP.

2.2 Foliar nutrition

Three foliar nutrients - seaweed extract Sagarika, nano urea, and water spray - were employed in the experiment. Nano Urea, a product based on nanotechnology, provides plants with nitrogen. It has a higher surface area and a suitable particle size of 20–50 nm compared to traditional urea prills. Following each harvest, 4 ml L^{-1} of nano urea was sprayed in the field. Sagarika, a concentrated seaweed extract, is an organic biostimulant that contains red and brown algae. The product was purchased from Indian Farmers Fertilizer Cooperative Limited. At a rate of four milliliters per litre, Sagarika was sprayed onto the field. The foliar applications were done after the first and second harvests at 90DAP and 135 DAP.

2.3 Observations

At 180 days after planting (DAP), the leaves were analyzed for indican, nitrogen (N), phosphorus (P), and potassium (K) contents. The indican content was determined spectrophotometrically at 280 nm following methanol extraction of the leaves (Wu *et al.,* 1999). Nitrogen and potassium contents were analyzed using standard procedures outlined by Jackson (1958), while phosphorus content was determined according to the method described by Piper (1966).

3. RESULTS AND DISCUSSION

3.1. Effect of treatments on indican content of *Indigoferatinctoria*

The impact of treatments on indican content in leaves is illustrated in Figure 1. The application of farmyard manure (FYM) at 10 t ha^{-1} significantly enhanced indican content (1.208%) in leaves, while the lowest content (1.111%) was observed with FYM at 5 t ha^{-1}. This finding is consistent with Sindhu *et al*. (2016), who reported increased indican content in indigo plants with FYM application at 10 t ha^{-1}.

A two-year pooled analysis revealed that NPK levels of 30:10:60 kg ha^{-1} and 40:10:80 kg ha^{-1} significantly increased leaf indican content. In contrast, the lowest indican content (1.128%) was observed with NPK at 10:10:20 kg ha^{-1}. This suggests a positive correlation between nitrogen, potassium, and indican content. An increase in the oil content of basil by the application of nitrogen was reported by Arabaci and Bayram (2004).

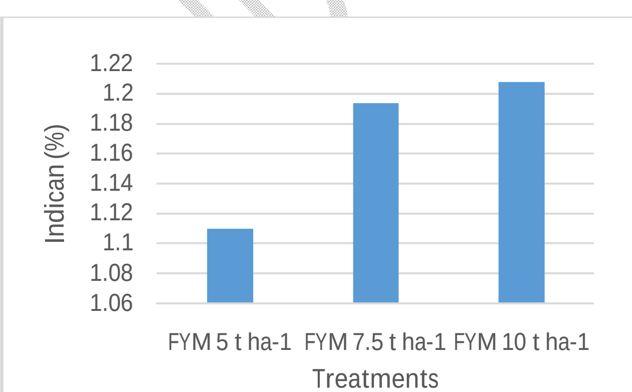
Foliar nutrition with seaweed extract (IFFCO Sagarika) at 0.4% significantly increased indican content (1.299%) in leaves compared to other stimulants. The lowest indican content was observed with water spray.

3.2. Effect of treatments on N,P,K contents of *Indigoferatinctoria*

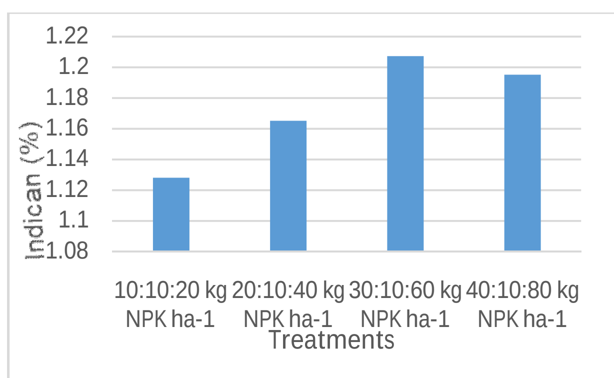
The effects of treatments on plants' nitrogen, phosphorous, and potassium content are depicted in Figure 2. The highest nitrogen content (3.162 %) in leaves was observed in plants, which received FYM 10 t ha^{-1} and was on par with FYM 7.5t ha^{-1}.The lowest nitrogen content (2.848%) was observed with FYM 5 t ha^{-1}. The phosphorus and potassium content of plants also showed the same conclusion.

Concerning the effect of fertilisers on the nitrogen content in leaves, a higher nitrogen value was observed in the treatment40:10:80 kg NPK ha^{-1}(3.146 %),which was on par with NPK level 30:10:60 kg NPK ha^{-1}. The lowest nitrogen content (2.956 %) was observed with 10:10:20 kg NPK ha^{-1}. The tendency was the same for the potassium content of plantsas well. The phosphorous content of the plant increased (0.363%) with NPK level 40:10:80 kg NPK ha^{-1},while the lowest phosphorous content (0.336%) was noted with NPK level 10:10:20 kg NPK ha^{-1}.

The application of nano urea enhanced the nitrogen content (3.174 %) of leaves. Nano urea is the nano-sized particle of urea. It contains a high surface area and high penetration capacity. IFFCO nano urea contains 1-5 % nitrogen (Kumar *et al*., 2021). A significant difference was observed between foliar nutrition and the phosphorous content of plants. Plants treated with seaweed extract had the highest phosphorus concentration (0.358%), while water spray treatments had the lowest (0.336 %). According to an experiment by Pramanick*et al.* (2014), the 15% solution of red algae *kappaphycus* and a recommended fertilizer dose had the highest phosphorus uptake by rice grain and straw.The leaves' potassium content increased after applying seaweed extract (2.665%), whereas the water spray had the lowest potassium content (2.505%).



Effect of different FYM levels on indican content



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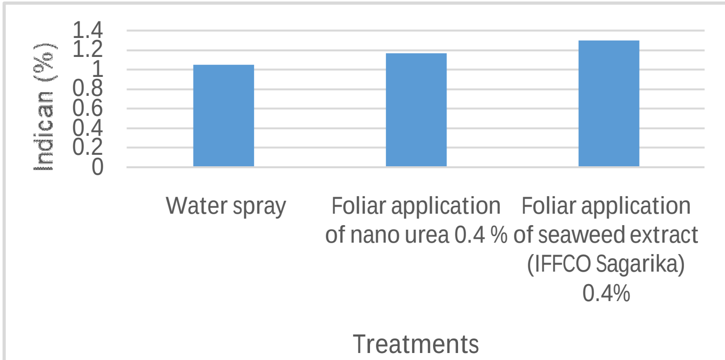
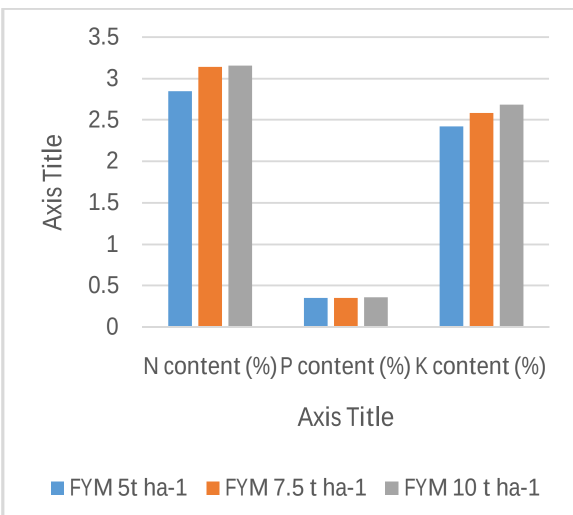
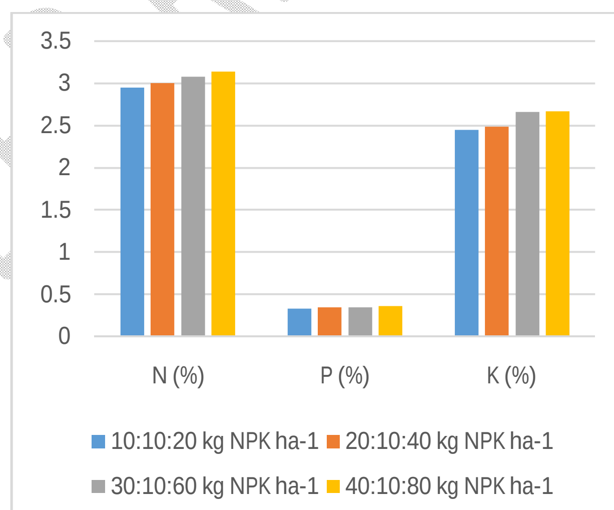


Effect of different foliar nutrition on indican content

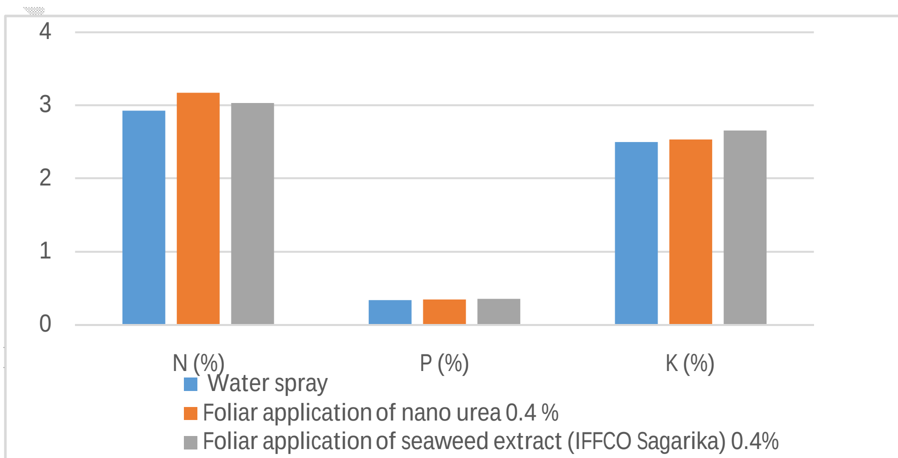
Fig. 1. The direct effect of treatments on indican content of *Indigoferatinctoria* at 180 DAP (pooled mean of two years)



Effect of different FYM levels on N,P,K contents



Effect of different NPK levels on N,P,K contents



Effect of different foliar nutrition on N,P,K contents

Fig. 2. The direct effect of treatments on nitrogen, phosphorus, and potassium contents (%) of *Indigoferatinctoria* at 180 DAP (pooled mean of two years)

3.3 Interaction effect of FYM, fertilisers, and foliar nutrition on indican, N, P, and K contents

The interaction effects of FYM, fertilizers, and foliar nutrition on the levels of indican, N, P, and K are statistically analysed and presented in Table 1. The interaction effect of FYM, fertilisers, and foliar nutrition was found to be significant for indican content, N, and K contents of leaves but non-significant for P content in leaves.

The indican content of leaves was enhanced (1.409 %) by the combined application of FYM 10 t ha^{-1}, 40:10:80 kg NPK $ha^{-1,}$ and seaweed extract and it was on par with all treatment combinations with seaweed extract except FYM 5 t ha^{-1} + 10:10:20 kg NPK ha^{-1}. All treatment combinations with nano urea also produced the same result except FYM 5 t ha^{-1} + 20:10:40 kg NPK ha^{-1} and FYM 5 t ha^{-1} + 10:10:20 kg NPK ha^{-1}.

Statistically analysedtwo-year data revealed that application of FYM 10 t ha^{-1} along with 40:10:80 kg NPK ha^{-1} and nano urea enhanced the nitrogen content (3.355 %) of plants and was on par with all treatment combination which includednano urea except FYM 5 t ha^{-1} + 30:10:60 kg NPK ha^{-1}, FYM 5 t ha^{-1} +20:10:40 kg NPK ha^{-1} and FYM 5 t ha^{-1}+10:10:20 kg NPK ha^{-1}.Applying seaweed extract along with FYM 10 t ha^{-1} and 7.5 t ha^{-1} with different combinations of NPK levels, except FYM 7.5 t ha^{-1} + 10:10:20 kg NPK ha^{-1},also produced the same result.

Combined application of FYM 10 t ha^{-1}, NPK level 30:10:60 kg NPK $ha^{-1,}$ and seaweed extract significantlyincreased the potassium content (3.006 %) of leaf and was on par with FYM 10 t ha^{-1} + 40:10:80 kg NPK ha^{-1} + seaweed extract and FYM 10 t ha^{-1} + 40:10:80 kg NPK ha^{-1} + nano urea. Improvement in potassium uptake of plants due to the application of 15% *Kappaphycus* sap along with the recommended dose of fertilizer was reported by Dwivedi *et al.* (2014). The lowest content of potassium (2.011 %) was observed in plots that receivedthe combined application of FYM 5 t ha^{-1} +10:10:20 kg NPK ha^{-1}+water spray.

Table 1. Interaction effect of FYM, fertilisers and foliar nutrition on indican, N,P, and K contents (%) of *Indigoferatinctoria* at 180 DAP (Pooled mean of two years)

Treatment combination	Indican content (%)	N (%)	P (%)	K (%)
FYM 5 t ha^{-1} +10:10:20 kg NPK ha^{-1}+Water spray	0.993	2.47	0.301	2.011

FYM 5 t ha^{-1}+10:10:20 kg NPK ha^{-1} +Nano urea	1.054	2.76	0.314	2.207
FYM 5 t ha^{-1} + 10:10:20 kg NPK ha^{-1}+ Seaweed extract	1.139	2.666	0.32	2.424
FYM 5 t ha^{-1} + 20:10:40 kg NPK ha^{-1} + Water spray	0.932	2.69	0.331	2.194
FYM 5 t ha^{-1} + 20:10:40 kg NPK ha^{-1} + Nano urea	1.015	3.035	0.332	2.357
FYM 5 t ha^{-1} +20:10:40 kg NPK ha^{-1} + Seaweed extract	1.182	2.856	0.332	2.495
FYM 5 t ha^{-1} + 30:10:60 kg NPK ha^{-1} + Water spray	1.021	2.835	0.336	2.487
FYM 5 t ha^{-1} + 30:10:60 kg NPK ha^{-1} + Nano urea	1.18	2.918	0.332	2.532
FYM 5 t ha^{-1} + 30:10:60 kg NPK ha^{-1} + Seaweed extract	1.294	2.863	0.35	2.608
FYM 5 t ha^{-1} + 40:10:80 kg NPK ha^{-1} + Water spray	1.062	2.907	0.343	2.54
FYM 5 t ha^{-1} + 40:10:80 kg NPK ha^{-1} +Nano urea	1.185	3.159	0.352	2.61
FYM 5 t ha^{-1} + 40:10:80 kg NPK ha^{-1} + Seaweed extract	1.275	3.015	0.359	2.593
FYM 7.5 t ha^{-1} + 10:10:20 kg NPK ha^{-1} + Water spray	1.088	3.005	0.331	2.581
FYM 7.5 t ha^{-1} + 10:10:20 kg NPK ha^{-1} + Nano urea	1.172	3.165	0.342	2.58
FYM 7.5 t ha^{-1} + 10:10:20 kg NPK ha^{-1} + Seaweed extract	1.313	3.023	0.351	2.593
FYM 7.5 t ha^{-1} + 20:10:40 kg NPK ha^{-1} + Water spray	1.146	2.91	0.342	2.57
FYM 7.5 t ha^{-1} + 20:10:40 kg NPK ha^{-1} + Nano urea	1.211	3.199	0.345	2.424
FYM 7.5 t ha^{-1} + 20:10:40 kg NPK ha^{-1} + Seaweed extract	1.356	3.078	0.364	2.448
FYM 7.5 t ha^{-1} +30:10:60 kg NPK ha^{-1} +Water spray	1.147	2.972	0.343	2.562
FYM 7.5 t ha^{-1} +30:10:60 kg NPK ha^{-1} + Nano urea	1.205	3.272	0.349	2.577
FYM 7.5 t ha^{-1} +30:10:60 kg NPK ha^{-1} + Seaweed extract	1.357	3.22	0.344	2.785
FYM 7.5 t ha^{-1} +40:10:80 kg NPK ha^{-1} + Water spray	1.045	3.018	0.361	2.553
FYM 7.5 t ha^{-1} +40:10:80 kg NPK ha^{-1} +Nano urea	1.199	3.29	0.361	2.544
FYM 7.5 t ha^{-1} + 40:10:80 kg NPK ha^{-1} +Seaweed extract	1.278	3.306	0.347	2.658
FYM 10 t ha^{-1} + 10:10:20 kg NPK ha^{-1}+ Water spray	0.985	3.023	0.339	2.512
FYM 10 t ha^{-1} + 10:10:20 kg NPK ha^{-1} + Nano urea	1.163	3.307	0.342	2.433
FYM 10 t ha^{-1} + 10:10:20 kg NPK ha^{-1} + Seaweed extract	1.242	3.135	0.377	2.715
FYM 10 t ha^{-1} + 20:10:40 kg NPK ha^{-1} + Water spray	1.016	3.025	0.357	2.619
FYM 10 t ha^{-1}+ 20:10:40 kg NPK ha^{-1} + Nano urea	1.243	3.238	0.362	2.582
FYM 10 t ha^{-1} + 20:10:40 kg NPK ha^{-1} + Seaweed extract	1.353	3.158	0.37	2.701
FYM 10 t ha^{-1} + 30:10:60 kg NPK ha^{-1} + Water spray	1.023	3.04	0.36	2.681
FYM 10 t ha^{-1} + 30:10:60 kg NPK ha^{-1} + Nano urea	1.254	3.33	0.361	2.754
FYM 10 t ha^{-1} + 30:10:60 kg NPK ha^{-1} + Seaweed extract	1.389	3.246	0.381	3.006
FYM 10 t ha^{-1} + 40:10:80 kg NPK ha^{-1} + Water spray	1.143	2.998	0.367	2.746
FYM 10 t ha^{-1} + 40:10:80 kg NPK ha^{-1} + Nano urea	1.259	3.355	0.381	2.843
FYM 10 t ha^{-1} + 40:10:80 kg NPK ha^{-1} +Seaweed	1.409	3.082	0.395	2.954

extract				
CD(0.05)	0.259	0.30	NS	0.1877
CV (%)	10.285	8.954	16.726	15.689

4. CONCLUSION

The two-year investigation conclusively demonstrated that farmyard manure, NPK levels, and foliar nutrition exerted a profound impact on the chemical and biochemical parameters of *Indigoferatinctoria* L. Specifically, the optimal treatment combination of FYM at 10 t ha^{-1}, NPK at 30:10:60 kg ha^{-1}, and foliar nutrition with 0.4% seaweed extract was identified as a recommended practice for enhancing the chemical and biochemical properties of the plant.

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