Potentialities of Indigo plant (*Indigofera tinctoria*) Production in the Sudan for Domestic Use, and Exportation

By

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Thesis

Submitted to the University of Khartoum in Fulfillment of the Requirement for the Degree of Doctor of Philosophy in Agriculture

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May ٢٠٠٦

DEDICATION

To my late father, mother and husband

ACKNOWLEDGEMENT

I would like to express my sincere thanks and appreciation to my supervisors Dr. Mustafa M. Elballa, Department of Horticulture, Faculty

of Agriculture, U. of K. and Dr. Hassan El Subki, Medicinal and Aromatic Plants Institute, National Center for Research, for the initiation of this work, their encouragement, guidance and continuous help throughout the study. Deep gratitude is due to Dr. Mohamed Abo, Sudan University of Science and Technology, for the initiation of this work, valuable guidance and suggestions during the planning of the outlines of the analytical part of this study. Thanks and appreciations are extended to staff of Shambat Research Station for their great assistance during this study. Appreciations are due to all technicians for their assistance. Appreciations are extended to Dr. Salah Hamed for his assistance in statical analysis. My thanks and appreciations are expressed to all those upon whose support and diligence to conduct this work. My thanks and wishes are expressed to my friends Zeinab and Amna for their tireless efforts and continuous help. My thanks are extended to those who cooperated and helped but not named here.

Last, but not least, my sincere thanks and gratitude to my mother and husband for their patience, unlimited help, and encouragement throughout this work.

ABSTRACT

Four field experiments were conducted for two growing seasons (٢٠٠١/٢٠٠٢ and ٢٠٠٢/٢٠٠٣) at Shambat Research Station (Sudan).

The objectives of the study were to determine the management practices needed for crop establishment in the field. The main objectives were to determine the optimum cultural practices affecting yield and dye content of the Indigo plant (Hennat elgroud) including the effect of growing season on the performance of the plant, the effect of sowing date and plant spacing, effect of water management and cutting frequencies and the effect of fertilizer application and stage of harvest. The performance of the plants was measured in terms of leaves dry weight and dye content.

In the first experiment, the seeds were sown in the winter season, in the summer and in the autumn season.

The second experiment comprised four monthly sowing dates, commencing in June as an early date up to September as the late sowing date. Three plant spacing were used ٧٥cm, ١٠٠ cm or ١٢٠ cm between plants.

The third experiment was designed to study the effect of three watering intervals (i.e. ٧-١٠ or ١٥ days) and the effect of watering stoppage at ٧-١٠ or ١٥ days before harvesting in combination with two cutting treatments. In the first treatment the plants were subjected to two cut, after one month from planting and a second cut after two months and in the second treatment, the plants were cut once after two months.

The fourth experiment, the Indigo plants were harvested at four stages: after one month from planting, before flowering, at full flowering and at fruit setting stage. Four different treatments of fertilizer were also studied, control, (no fertilizer), composted chicken manure at the rate of

٢,٥ tons /fed, nitrogen fertilizer in the form of urea "٤٦٪ N," at the rate of ٥٠kg/fed and ١٠٠ kg/fed.

In the winter season, the seeds failed to germinate and the results of this season were excluded.

The results of the combined analysis between the summer season and the autumn season showed the total yield obtained in the summer season was more than ١٢,٨٪ of the autumn season. The total dye content obtained from the summer season out-yielded the dye content obtained from the autumn season by ٩,٥٪.

The results of the second experiment showed that in both seasons, the total yield and total dye content decreased progressively with delayed sowing. The highest yield and dye content were obtained from the closest plant spacing (٧٥ cm).

The results of water management in both seasons showed that the yield and dye content were increased with decreasing irrigation intervals. The effect of watering stoppage showed that yield and dye content were decreased by increasing time of watering stoppage before harvest. The results of the effects of cutting treatment on yield and dye content showed that the plants subjected to two cuts, out-yielded the plants subjected to one cut after two months from planting.

In the fourth experiment the results showed that harvesting at full flowering stage produced the highest total yield in both seasons. Similar results were obtained in the dye content. Addition of Nitrogen fertilizer at ١٠٠ kg/fed gave the highest yield and dye content, and the control gave lower yield and dye content than the rest of the treatments in both seasons.

The extraction of dye content was investigated using different solvents. The results showed that the highest dye content was obtained by using ethanol (٨٠٪). The most suitable solvent systems for separation were Benzene**:** Petroleum ether at ratio of ٢٠:١ and Benzene: Ethyl acetate at ratio of ٨٠:٢٠.

TLC technique showed that the dye content possesses more than three components according to R_f values and color of bands. The colors ranged between blue- violet and R_f ranged between ٠,٢٤-٠,٨٣.

ملخص الأطروحة

أجريت أربع تجارب حقلية خلال موسمى ٢٠٠١/٢٠٠٢ و٢٠٠٢/٢٠٠٣ بحقل محطة بحوث شمبات (السودان) .

تهدف هذه الدراسة الى تأسيس المعلومات الزراعية المطلوبة لانتاج نبات النيلة (حنة القرود) . الهدف الرئيسى من الدراسة هو تحديد المعاملات الزراعية التى تؤثر على الأنتاجية والمحتوى الصبغى للنبات.

وتشمل الدراسة:

١- تاثير مواسم الزراعة على النمو.

٢- تاثير مواعيد ومسافات الزراعة.

٣- تاثير فترات الرى وفترات ايقاف الرى قبل الحصاد وتكرار قطع النبات.

٤- تاثير مواعيد الحصاد واضافة السماد.

تم قياس الوزن الجاف للاوراق والمحتوى الصبغى.

فى التجربة الأولى زرعت البذور فى موسم الشتاء, موسم الصيف و موسم الخريف.

شملت التجربة الثانية أربعة مواعيد زراعة وكانت الزراعة شهرياً ابتداءً من يونيو كزراعة مبكرة وحتى سبتمبر كزراعة متأخرة. أيضاً شملت ثلاث مسافات زراعة ٧٥-١٠٠ -١٢٠ سم بين النباتات.

صممت التجربة الثالثة لدراسة تأثير ثلاث فترات رى (٧-١٠ -١٥ يوم) وثلاث فترات ايقاف الرى قبل الحصاد (٧- ١٠- ١٥ يوم) وخضعت النباتات للقطع بعد شهر من الزراعة ثم بعد شهرين من القطعة الأولى ونباتات خضعت للقطع مرة واحدة بعد شهرين من الزراعة.

فى التجربة الرابعة حصدت النباتات فى أربعة مراحل وهى بعد شهر من الزراعة – قبل الأزهار- عند اكتمال الأزهار- ومرحلة عقد الثمار. ايضاً شملت التجربة أربع معاملات تسميد وهى معاملة الشاهد (لا تسميد) – ٢,٥ طن للفدان مخلفات دواجن - -اضافة سماد اليوريا بمعدل ٥٠ كيلو جرام للفدان أو ١٠٠ كيلو جرام للفدان.

تم إلغاء الزراعة في فصل الشتاء نسبة لضعف نسبة الأنبات.

أوضح التحليل الذى يضم نتائج موسمى الصيف والخريف أن الأنتاجية الكلية في موسم الصيف أكبر من الأنتاجية الكلية فى موسم الخريف بحوالى ١٢,٨ %. كذلك كان المحتوى الصبغى الكلى المتحصل عليه بالزراعة فى موسم الصيف أكبر من المتحصل عليه بالزراعة فى الخريف بحوالى ٩,٥ % .

فـى التجربـة الثانيـة فـى الموسـمين كانـت الأنتاجيـة الكليـة والمحتـوى الـصبغى المتحصل عليهما بالزراعة المبكرة فى يونيو أعلى من المتحصل عليهما بالزراعة المتأخرة فـى سـبتمبر. أعطـت مـسافة الزراعـة الـضيقة ٧٥ سـم بـين النباتـات أعلـى أنتاجيـة ومحتـوى صبغى فى الموسمين.

أظهـرت فتـرات الـرى تـأثيراً معنويـاً علـى الأنتـاج والمحتـوى الـصبغى فـى الموسـمين حيث أتضح أن الرى كل ٧ أيام أدى الى حدوث زيادة معنوية على الرى كل ١٥ يوم.

كان تأثير فترة ايقاف الرى معنوياً علـى الأنتـاج والمحتـوى الـصبغى فـى الموسـمين حيـث أدى ايقاف الرى قبل ٧ أيام من الحصاد الى زيادة فى الأنتاجية والمحتوى. أتضح من نتائج التجربـة أن النباتات التى خضعت للقطع مرتيين أعطت أعلى أنتاجية ومحتوى صبغى والنباتـات التـى خضعت للقطع مره واحدة بعد شهرين من الزراعة أعطت أقل أنتاجية ومحتوى صبغى.

فـي التجربـة الرابعـة أعطـت مرحلـة الحـصاد عنـد تمـام الأزهـار أعلـى أنتاجيـة ومحتوى صبغى وإضافة سماد اليوريا بمعدل ١٠٠ كجم/للفدان أعطـى أعلـى أنتـاج ومحتـوى صبغى بينما أعطى الشاهد أقل أنتاج ومحتوى صبغى فى كلا الموسمين.

الاسـتخلاص بمحاليـل مختلفـة أوضـح أن أعلـى محتـوى صـبغى كـان عنـد الاسـتخلاص باسـتعمال الايثـانول (٨٠ %) . أمـا أنـسب نظـام محلـولى لفـصل الـصبغة كـان باستخدام البنزين: البتروليم ايثر بنسبة ١:٢٠ كذلك البنزين: الايثايل اسيتيت بنسبة ٢٠:٨٠ .

أظهر الفصل باستخدام طبقات الكروماتوغراف الرقيقة أن الصبغة تحتوى على أكثر من ثلاث مكونات حسب لونها وقيمة R_f .

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CHAPTER ONE

INTRODUCTION

Indigo, the blue dye has been known and used throughout the world in many countries; it has been used since Neolithic time in Europe, and was highly prized for its color and light fastness. The history of indigo goes much further back, it has been found on the bandages of mummified bodies taken from grave sites that dated as early as ١٥٨٠BC.

Indigo is the common name for a blue pigment and the word indigo means "a blue color". Indigo is the modern English name, however, there are several names by which the ancient Greeks and Egyptians also referred to indigo, n-tinkon in Egypt and Inditon in Greece. In China it is called lancoa, in Japan awa ai, and seitai, in India the word for indigo was nilah, meaning dark colored or black hue and in Sudan the local name is hennat elguroud.

Indigo refers to several species of *Indigofera*, a genus of leguminous plants, there are some ٧٠٠ species scattered throughout the tropical and sub-tropical countries. However, the most important is French indigo "*Indigofera tinctoria* L" an old world species, and Guatemala "*Indigofera suffruticasa* M" which was classified as *Indigofera anil*. L., a new world species. The other dye plants were *Isatis tinctoria* the woad, the sole source of indigo and Chinese indigo "*Isatis indigotica*, a kind of woad, and *Polygonium tinctorium* in temperate climates, and the American indigo *Baptisi tinctoria* which yields a poor

quality of indigo. There are some species of the genus *Indigofera* reported in the different parts of the Sudan.

Up to now, natural dyes have been neglected as renewable resources for agriculture, although there is an increasing demand for natural textiles produced from fibers, wool, cotton, linen, etc. Due to the development of the cheaper synthetic colors, natural dyes were replaced and cultivation of dye plants nearly ceased. An artificial product, indigotine, is manufactured chemically and was produced in ١٨٩٧ by Badishe Anilin Soda Fabric.

Nowadays interest in natural dye stuffs has revived. This is due to the awareness that synthetic dyes are principal source of environmental pollution, and they have several carcinogenic properties and cause allergy in humans. During the last few years, there has been a resurgence of interest in natural dyes to replace synthetic dyes.

Natural dyes, such as indigo are least toxic. Only a small percentage of the synthetic pigments have been tested for toxicity or long-term hazards.

Indigo is a highly priced commodity. It was a major dye used for fabrics, mostly dye Jeans. It can be used alone, or used in combination with other dyes, to produce a wide range of colors.

Indigo has been used alone or in combination with henna for dying the hair in black for at least ٤٠٠٠ years. During the ١٩th and ٢٠th centuries indigo was a common hair-dye in Europe and it was marketed as black henna. If you open a box of "black henna" that is indigo, you see green powder with a color and smell like frozen peas; if you mix with water you will see a blue glaze from the surface, and if you open a box of

"black henna" and the powder is brownish black or black it probably has "Para-phenylene, diamine (PPD)" in it.

Skin painting; using henna, is part of religious, social and ritualistic traditions in many parts of the world, henna is one of the most frequently used dyes. Common red henna (*Lawsonia inermis*, family Lythraceae) contains the active ingredient napthoquinone, which is responsible for the red- brown dye.

As a result of the negative impact of using the poisonous PPD, and the worrying trend towards the use of PPD as hair dye, the company (Tag Cosmetics LTD) decided to produce an alternative product, hennat el Guroud which is packed similar to henna.

Regarding the agricultural practices, no proper research has been carried out on the indigo plant. Due to the importance of this plant, the research reported in this thesis has been designed and executed to answer some of the basic questions about the husbandry of the plant for domestic uses, industrialization and exportation.

FAO (١٩٩٥) reported a paucity of reliable information in the literature on the indigo plant. Also (Shewry**,** *et al.,* ١٩٩٧) reported that due to the lack of commercial interest there has been no systematic scientific research on the plant as an economic alternative source of dyes for industrial purposes.

The main objectives of the present study were to determine the cultural practices affecting yield and dye content including:-

١. Evaluation of the effect of growing season on the performance of Indigo plant.

٢. Investigation of the effect of sowing date and plant spacing on yield

and dye content.

٣. To evaluate the effect of watering intervals, watering stoppage and cutting frequencies on crop yield and dye content.

٤. To study the effect of fertilizer application and stage of harvest on yield and dye content.

CHAPTER TWO
LITERATURE REVIEW

٢,١ Botany:

٢:١:١ Leguminosae:

The leguminosae is the third largest family of all flowering plants. It has a world wide distribution and currently is estimated to contain ١٦٠٠٠ species in about ٧٥٠ genera. Nearly one third of the species belongs to six genera: *Acacia, Astragulus, Cassia, Crotalaria, Indigofera,* and *Mimosa.* Leguminales is an order, which include three families: Caesalpiniaceae, Mimosaceae and Papilionaceae. Other botanists considered the Leguminosae as a single family comprising three sub-families: Mimosoideae, Caesalpinodeae and Papilionideae (Khalid, ١٩٩١).

Leguminosae includes more important drugs than any other family. It is divided into three subfamilies, the Papilionideae, Mimosoideae, and Caesalpinodeae containing about ٣٧٧- ٤٠ and ١٣٣ genera, respectively, (Trease and Evans, ١٩٧٨). It comprises an important plant source supplying vegetable protein to human and animals and the greatest

variety of leguminous plants is found in the tropics and subtropics (Thompson and Kelly, ١٩٥٩).

٢:١:٢ Sub-family Papilionaceae:

The Papilionaceae family includes herbs, shrubs and trees. Leaves are simple or compound. Flower is zygomorphic and papilionaceous, with ١٠ stamens, monadelphous or diadelphous. Fruit is a legume. The most important genera are, *Lupinusu* (٢٠٠ species) *Medicago* (١٠٠ species), *Trifolium* (٣٠٠ species), *Indigofera* (٧٠٠ species) Astragalus (٢٠٠٠ species), *Vicia* (١٥٠ species) Phaseolus (٢٠٠-٢٤٠ specie) *Trigonella* (١٠٠ species) and *Crotalaria* (٥٥٠ species), (Trease and Evans, ١٩٧٨).

٢:١:٣ Genus Indigofera

This genus has ٧٠٠ species (http://www.). *Indigofera* contains annual, and perennial herbs, shrubs, and small trees with pinnate or trifoliate leaves, racemes or spikes of small pea-like flowers (Abd Almula, ٢٠٠٠). *Indigofera* species became an important cash crop in various tropical and subtropical areas. The majority occurs in Africa (٥٢٠ species) with other centers of diversity in Arabia to South East Asia, subtropical North and South America (Brian, *et al*., ١٩٩٧). It contains important dye plants, some species are grown as green manure, fodder, and used as cover crop, especially in tea, coffee, rubber plantations and a few are grown as ornamentals (http://www.) .

٢:١:٣: ١ *Indigofera oblingofolia:*

Shrubs ١-٣ m high, branches stiff gray silvery or red brown, stipules persistent triangular, pubescent leaflet oblong elliptic, ١- ٢,٥ cm glaucons flowers scerel, fruit ٦-٨ seeds, pod ٢ cm long – brown red, tometose constricted. It is widely spread in north and central Sudan along river beds in short grass savanna (Abd Almula, ٢٠٠٠).

Uses:

The fumigant of the whole plant was used with Sesame oil as anti-rheumatic (El Ghazali, *et al*., ١٩٩٤). An antibacterial action of the small protein peptide obtained from fractions of the leaves were tested against *Eftherithia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, and showed a strong inhibitory activity against *B. subtillus* and *Aspergillus niger* (Umar Dahot ١٩٩٩). In Yemen it is used by traditional healers to treat infectious diseases and was screened for its antibacterial activity against both Gram positive and Gram negative bacteria, and promising results were obtained from the ethanolic extract (Ali, *et al.,* ٢٠٠١).

٢:١:٣: ٢ *Indigofera hirsuta:*

Erect or decumbent herbs, up to ١ m high, stems, especially young parts, densely clothed with long fine spreading grey pubescence, leaves are compound of ٥-٧ leaflets, obovate and the terminal is the largest, ١,٧x١,٠cm, grey silk above, densely so beneath. Inflorescences are dense, lax auxiliary racemes, up to ١٨ cm long, flowers with scarlet to pinkish corollas, fruits straight slightly deflexed pods, densely covered

with grey or brown pubescence, ٦-٨ seeds, up to ٢ cm long (ElGhazali, *et al.*, ٢٠٠٣).

Uses:

In the folk medicine in the Sudan, the infusion of the roots in sesame oil is used as anti asthmatic (ElGhazali, *et al.*, ٢٠٠٣).

٢:١:٣:٣ *Indigofera arrecta:*

Erect branched undershrubs, up to ١ m high, stems sulcate, grey-pubescent with medifixed hairs, leaves compound, alternate, leaflets ١٣, obovate-oblanceolate, obtuse and emarginated at apex, up to ٠,٩-١,٨ cm, both surfaces subglaucous, nearly glabrous above, with short medifixed hairs beneath, inflorescences auxiliary racemes, corollas red, fruits cylindrical pods, slightly constricted up to ٣ cm long, shortly peaked, thinly pubescent, (ElGhazali, *et al.*, ٢٠٠٣).

Uses:

It is used as blue dyestuff for textiles; *I. arrecta* is regarded as much superior to *I. tinctoria* and farmers have largely supplanted in India (FAO document ١٩٩٥). In Sudan, traditionally the maceration of the roots is used against dysentery and gonorrhea, (ElGhazali, *et al.*, ٢٠٠٣).

٢:١:٣:٤ *Indigofera tinctoria*:

Shrubby herbaceous plant , up to ١,٥ m high, but usually less than ١ m. Stem erect, copiously branches often widely spreading; leaves compound, alternate, leaflets ٧-١١, oblong or obovate, ١٠-٢٧mm long; ٤-١٧mm broad; sub glabrous, more or less glabrous, inflorescence axillary

racemes, corollas purplish, pods glabrescent, ١٥-٣٥mm long, about ٢mm wide up to ٣,٥ cm long, glabrous, deflexed, ٣-١٥ seeds, usually ٦-١٢, much longer than broad (Andrews ١٩٥٢, Duke, ١٩٨٣ and El Ghazali, *et al*., ٢٠٠٣,) (Fig ١).

٢:١:٤ Germplasm

No breeding or selection has been undertaken for the Indigo plant. It is cross-pollinated by insect. *Indigofera tinctoria* (٢n=٢x=١٦), *I. arrecta* (Africa, ٢n=٢x=١٦), *I. anil* (Southern-Asia, ٢n=٢x=١٢) Simmonds, (١٩٧٩).

Fig (١) *Indigofera tinctoria*

Whole plant

Branch

Pods

٢:١:٥ Distribution

Native to the Malaysian, it grows spontaneously in Africa. It was introduced and widely cultivated in India, China, Java, Africa, Malagasy and tropical America (Duke ١٩٨٣).

٢:١:٦ Ecology

Indigo is widely cultivated in tropical regions, from sea level to ٣٠٠ m. It grows well along river banks, roadsides and brushwood and grassy field. Excessive heat and hot winds cause withering. In the US, it was restricted to hardiness zone; ranging from warm temperate through tropical dry to wet forest life zone. It tolerates annual precipitation of

٦,٤-٤١,٠ dm, annual mean temperature of ١٠,٥-٢٧,٤ °c and pH of ٤,٣-٨,٧(Duke, ١٩٨٣).

٢:١:٧ Uses:

Indigo plant is cultivated for the blue dye. In southern India, it is used as a cover or green manure crop in coffee plantations and rice field. Leaves are rich in potash and the plant is said to be palatable to cattle (Duke ١٩٨٣). The Chinese used it to clean the liver, detoxify the blood, reduce inflammation, alleviate pain, and reduce fever (Simon, *et al.*, ١٩٨٤). Plant compounds used in traditional Chinese medicine consist of extract from *Indigofera* leaves was used for leukemia treatment and lung cancer (Han, ١٩٨٨). Chronic myelocytic leukemia has customarily been treated with traditional Chinese recipe, Danggui Longhui Wan, a mixture of eleven herbal medicines. Antileukemic activity was attributed to the red colored isomer of indigo, indirubin or indigo red (Ralph, *et al.*, ١٩٩٩). Methanol extraction of whole plants of *Indigofera tinctoria* was screened for activity as inhibitors of HIV-I (IIIB) and HIV-٢(ROD), the extracts exhibited activity of ١١٣ and ١٢٥ μ/ml, respectively (Kavimani, *et al.*, ٢٠٠٠). A bioactive fraction, indigtone (FA), obtained by fractionation of petroleum ether extract of the aerial parts of *I. tinctoria*, showed significant dose –related

hepatoprotective activity against CC١٤ induced liver injury in rats and mice (Singh, *et al.*, ٢٠٠١). In the Sudan it is used in folk medicine that the fresh roots are chewed against intestinal spasms (El Ghazali, *et al.*, ٢٠٠٣).

A study initiated to screen ٢٠ plants extracts for their efficacy against *Radopholus similis* attacking banana, showed that the roots extract of *I. tinctoria* exhibited a high degree of nematostatic action against nematode adults and Larva (Sreeja and Charles, ١٩٩٨).

Other uses of Indigo plant include its uses as nitrogen catch crops. Raj and Jagdisha (٢٠٠٢) mentioned that nitrate levels in ground water have been increasing over recent decades in most countries as a result of excess use of fertilizer to feed increasing population. The input-intensive cropping system has resulted in a problem of a large leakage of N into the environment, thereby polluting the water. Growing catch crop like Indigo plant and corn (*Zea mays* L.) reduced residual nitrate level up to ٦٨٪. A suggestion for developing indigo plant plus maize N catch crop in rotation was made to decrease nitrate leaching and maximize N use efficiency in rice and sweet pepper cropping system (Shrestha and Ladha, ١٩٩٨). In Cuba, Menendez, *et al.,* (١٩٩٥), reported that *I. tinctoria* was used as forage crop. Indigo plant was used as wild host plant for the pentatomid pest of legume crops, the green sting bug (*Nezara viridula*) (Antonio, ١٩٩٧).

The potential of legume green manure (Gm) as an alternative to mineral nitrogen fertilizer in tropical horticulture has received scant attention. The feasibility of meeting nitrogen needs to tomato with green manure was studied in Taiwan and Philippines, the yield response to green manure nitrogen is high on infertile soils and tomato nitrogen requirement can be substituted fully or partially by Gm, depending on soil mineralization, (Thonnissen, *et al*., ٢٠٠٠). *Indiogefera tinctoria* was evaluated as green manure crop, to test its efficiency in biomass

production, improvement of soil and substitution of nitrogen on the follow-up sugarcane. Total N, P and S, status of the soil increased slightly on use of these green manure crops, (Alam and Islam ١٩٩٧). In Philippines project program, (services per type of technology) reported that *Indigofera tinctoria* was used as a source of manure for soil improvement, producing large amounts of biomass, and the impact on poor women was evaluated. The results showed that it reduced the uses of chemical fertilizer, increased net income, and increased rice grain (http://www.). This was confirmed by Agustin, *et al.*, (١٩٩٨) who reported that farmers used indigo plant to replace and supplement chemical fertilizer and to improve soil fertility, experiences and research results showed higher rice gracing yields in fields previously planted and incorporated with indigo plant than those without indigo.

٢:٢ Husbandry

Some useful dye plants already exist as cultivatable crops but the majority of species would need selection from wild types to produce acceptable crop. Husbandry techniques would also need development, e.g. Pests, diseases and weed control, planting density, and harvest and post-harvest techniques (Hancock, ١٩٩٧).

FAO (١٩٩٥) reported a paucity of reliable information in the literature on the indigo plant. Also (Shewry**,** *et al.,* ١٩٩٧) reported that due to the lack of commercial interest there has been no systematic scientific research on the plant as an economic alternative source of dyes for industrial purposes.

٢:٢:١ Climate and soils

Indigofera tinctoria prefers sand loamy soils. It requires drained soil and will not thrive in clay. The plant grows better in neutral and alkaline soils, and it is known to tolerate very alkaline conditions (http://www.). It was reported that growth is best on permeable soils which are rich in organic matter (FAO, ١٩٩٥). On the other hand, experiment with thornless henna (*Lawsonia inermis*) and *Indigofera tinctoria* in hydroponics soil are studied. Leaf productivity and dye out put was more than ٣ times greater with soilless cultivation than soil (Mairapetyan, ١٩٨٨). Most of the commercial *Indigofera* species are adaptable to average of climate in the tropics and warmer areas of the sub-tropics, but display differing performance. *Indigofera jucanda* in South Africa is grown in summer with moderate to high rainfall, the best results were obtained from growing in semi-shade to full sun, and it grows in sandy to clay soils, (Brian, *et, al.,* ١٩٩٧). Gufu, *et al.,* (٢٠٠٠) reported that, *I. spinosa* indigenous to northeastern Africa, respond to climate variability. Khazhakyan, *et al*., (١٩٨٦) found that *I. tinctoria* and *I. articulate* grew better and were more productive in longer days (١٨h), and that *I. tinctoria* and *I. articulate* gave five times and twice higher leaf yields respectively than in short days (٨h). *Indigofera* can be grown at ٤٠٠-١٤٠٠m.a.s.L. leaves varied greatly depending on locality (Rasulova, *et al*., ١٩٨٦). On the other hand, Angelini *et al.,* ٢٠٠٤ reported that in rainfall conditions very high indican content and a potential high indigo yield can be obtained by cultivation of *Polygonum tinctorium*.

٢:٢:٢ Propagation

Propagation of indigo plant is usually by means of seed, and pre-soaking in water can assist germination. Abdi and Barker, (١٩٨٧) found that soaking seeds in hot water significantly improved both the percent germination and mean germination time. *Indigofera anil* seeds from a Mexican collection gave more than ٩٠٪ percent germination when a mechanical scarification was used (Moreira, *et al*., ١٩٩٤). This result was confirmed by Sy, *et al*., (٢٠٠١) who reported that manual scarification and immersion in acid increased germination capacity, germination was significantly higher at ٣٥°c. Seeds germinated in ٤-٥ days (Duke, ١٩٨٣). Stem cuttings were unsuccessful under any treatments of growth regulators, however, root cuttings of the largest girth showed the best rooting response (Philip, *et al.,* ١٩٩١).

٢:٢:٣ Sowing date

Indigo is sown at the commencement of the hot season February – March (http://www.) Also, In Bangladeshi, Indigo plant is cultivated during March-April (http://www.) *Indigofera anil* L, was sown at the beginning of the rainy season (http://www.) and in Japan, indigo plant seeds are planted in late winter and small plants are transplanted in April (http://www.). *Polygonum tinctorum* is sown in May in Germany (Anna and Christian ٢٠٠٣).

In other leguminous plants, Yamaguchi (١٩٨٣) reported that growth of snap bean is affected by temperature, and that temperature

below or above the main daily temperature (١٥-٣٠ ºC) can give a deleterious effect on performance of the plant. Ahamed (١٩٧٨) reported that Fadlalla and Burhan working on Clitoria, phillipesara and lubia sowing dates showed that for Clitoria and phillipesara, June sowing date gave the highest yield, where Lubia showed a rising trend in yield from July up to September where it gave its maximum yield and then dropped sharply by October. *Cassia angusifolia*, the medicinal plant which belongs to the family legumiosae is planted in June and July in Western India. (http://www.). Also in India, delayed sowing of soybean from June to July reduced the days to flowering, plant height, and number of branches (Ahmed, ١٩٧٨). In Sudan, Ahmed (١٩٨٦) mentioned that there was progressive improvement in seed quality attributes as sowing date between May and June.

٢:٢:٤ Plant spacing

Spacing is an important factor influencing the growth and development of crops and ultimately the yield. FAO document (١٩٩٥) reported that ٥٠-٦٠ cm spacing is common in *I. tinctoria*. Duke (١٩٨٣) and Anna and Christian, (٢٠٠٣) reported that seed drilling of dye plants has shown to be cheaper than planting.

Under Sudan conditions, some leguminous plants, phillipesara and hyacinth, produced higher forage yields per unit area of land at narrow spacing. In fodder bean, high densities are usually advantageous, with higher plant population, faba bean produced higher straw and dry matter yield (Elamin, ١٩٨٦). Close spacing between and within rows

significantly increased the biological and agricultural yield of cowpea, and in lubia closer spacing increased yield but differences did not reach significant level (Ahmed, ١٩٧٨).

٢:٢:٥ Fertilizers application

Under Sudan conditions, some leguminous plants were studied to determine the effect of nitrogen fertilizer on growth and yield. Mohamed (١٩٩٠) reported that nitrogen fertilizer significantly increased the yield of legume fresh and dry weight and growth attributes. This was confirmed by Rabih (١٩٩٩), who reported that nitrogen fertilizer significantly increased yield components of haricot bean. Nayel (١٩٨٤) studied the factors affecting Lucerne (*Medicago sativa* L.) production in Sudan, and reported that nitrogen fertilizer gave more fodder yield than the control. Mohamed (١٩٨٤) found that better growth of seedlings is expected when nitrogen compound is added as starter, growth and yield of cowpea plants responded to nitrogen application during early stage of growth and also at flowering. Also snap beans were very responsive to applied nitrogen and were less responsive to phosphate and potash (Mohamed, ١٩٧٦). Nitrogen fertilizer resulted in the highest yield of leaves from *Cassia acutifolia* (Elamin, ١٩٩٨) and ٨٠ kg of N was recommended (http://www.)

On the other hand, Gobara, (١٩٨٨) mentioned that nitrogeneaous fertilizers to snap bean is common practice, and doses as high as ١٠٠/kg /ha are recommended to obtain reasonable yield.

The organic cultivation of dye plants for the certified natural textiles industry is an emerging and promising sector of organic farming. The cultivation of dye plants in organic farming showed promising results, improvement of yield and quality, and development of dye extract (Anna and Christian ٢٠٠٣).

٢:٢:٦ Harvesting

In Japan, the plants are harvested in July for the first time, followed by a second harvest in August; the harvested leaves are spread out on an open area under the strong summer sun for one day and then mixed. After the leaves have been dried, the leaves and stems are separated. The leaves are further dried and used to make indigo (http://www.)

FAO document (١٩٩٥), reported that the first harvest is taken after three or four months from sowing and this involves cutting the stem ١٠-٢٠ cm above ground level, and that three cuts are obtained per year. Woad plant, *Isatis tinctoria* was harvested by a mechanical cutting, at least four times per year (http://www.). In the Ararat valley of Armenia, *I. tinctoria* and *I. articulata* are grown as annual crops, harvested once or twice depending on seasonal conditions and leaves from the first harvest had maximum indigo content (Mairapetyan, *et al.,* ١٩٨٦).

The same harvesting equipment that is used in herb and spices can be used for dye plant (Anna and Christian, ٢٠٠٣). Hancock, (١٩٩٧) reported that the quality of the dye colorant was variable through the season and with the age of the plant. Angelini, *et al.*, (٢٠٠٣) reported that

the growing season of *Polygonum tinctorium* is May- October and that indican increased a long the growing season until flowering and was positively affected by photosynthetic active radiation. Zavatskaya and Mashanova, (١٩٧٨) studied the sugar synthesis during plant development in relation to accumulation of the glycoside form of the dye; they found that sugar synthesis was maximal during full bloom when dyeing ability of the leaves was also highest. Cano, *et al.,* (١٩٩٠), reported that in *Cassia* the optimum glycoside constituents occurred when the plant is ٦٥ days old and decreased when plant age is ١٥٠-١٦٠ days. When woad plant was harvested in beginning July, end and beginning September, the highest yield and indigo dye was obtained when the plants were harvested in the end of July (http://www.).

٢:٢:٧ Irrigation

More than one irrigation of young plant is necessary (FAO, ١٩٩٥). Brian, *et al.,* (١٩٩٧) cited that *I. jucunda* is kept well watered during the summer months in South Africa with gradual easing off during the winter. Sy, *et al.*, (٢٠٠١) reported that the lowering of water potential diminished capacity of seed germination. Duke, (١٩٨٣) mentioned that irrigation should be carefully controlled and that too much water may kill the plants. In Sudan, according to Salih (١٩٩٢), many researchers reported that faba bean greatly benefited from watering at ٧ days intervals until maturity. This is in line with Abdalla (٢٠٠٢) who mentioned that increased watering intervals decreased growth attributes and yield components. Also Ahmed, (١٩٨٨) reported that extended

irrigation reduced both vegetative and reproductive attributes such as main stem length, number of leaflets and leaf area index of cowpea. In another leguminous crop, broad bean, Elamin (١٩٨٤) found that flowering phase is more sensitive to drought compared to the phase of pod development with greater reduction in attributes of vegetative and reproductive growth. Suliman, (٢٠٠٠) found that water stress in cowpea during vegetative stage reduced the number of days to first reproductive bud, while water stress during reproductive stage hastened flowering. Also the effect of drought at different developmental stage on yield and yield components of faba bean had been studied by Mohamed, (٢٠٠٣) who found that yield components were affected by water stress at most stages of development, drought stress reduced plant height, number of reproductive and vegetative branches, and date of flowering

٢:٢:٨ Weed control

Weed control in dye plants is necessary especially in the early growth stages, for conventional cultivation methods no herbicides are legally used (Anna and Christian, ٢٠٠٣). Duke, (١٩٨٣) reported that weeding starts when the plants are ٥-٨cm tall, with a small cutting tool and was continued until the crop is tall enough.

٢:٢:٩ Pests and Diseases

Extensive wilting and drying of *Indigofera tincroria* were shown in India due to infestation by Psyllid *Arytaina punctipennis*. (Skaria, *et, al*., ١٩٩٦). Indigo plant was attacked by fungi, susceptible to blight, green

caterpillars, locusts and other insects that feed on the leaves and flowers when plants are young. Plants are also attacked by nematode *Heterodera glycines* (Duke, ١٩٨٣).

٢:٣ Dyes

٢:٣:١ Definition and types of dyes

Dyes have been defined as intensely colored substances used for coloration. They are retained in substances by physical adsorption, mechanical retention, the formation of covalent chemical bonds or of complexes with salts or metals, or by solution. Pigments retain their crystalline or particulate structure throughout their application. Dyes lose their crystal structures during application by dissolution or vaporization. In the literature and common usage, the term dye and pigment tend to be used rather loosely and interchanged, but dye is often used for textile and food colorants, and pigment for inks, pains and cosmetic (Hancock, ١٩٩٧). Others define dye as; a usually soluble substance for staining or coloring e. g. fabrics or hair, synonym is dyestuff. Dyes are usually used as an aqueous solution and may require a mordant to improve the fastness of the dye on the fiber, they have an affinity to substrate to which they are being applied. In contrast the pigment generally has no affinity for the substrate, and is insoluble (http:// www.).

٢:٣:٢ Classifications

Dyes can be classified in two ways; by chemical composition, principally used by manufacturers and by application class or end use, principally used by dyers. Each dye is described under the internationally accepted Color Index classification system by a Cl Number, relating to its chemical class, and by a Cl Name, relating to use (Hancock, ١٩٩٧).

٢:٣:٣ Colorants Types

٢:٣:٣:١ Synthetic dyes

The first recorded synthetic dye is picric acid, which was produced in ١٧٧٠ from the interaction of indigo and nitric acid. The synthetic dye industry is considered to have started when Perkin synthesized Mauveine (Mauve) in ١٨٥٦ in the U.K. The industry quickly developed mainly in Germany, Switzerland and the U.K. Since then, the significant dyes discovered were alizarine red, indigo blue, and anthraquinone vat dyes. There are many synthetic dyes available now (Hancock, ١٩٩٧). In ١٨٦٥, the German chemist Johann Friedrich von Bayer began working with indigo. His work culminated in the first synthesis of indigo in ١٨٧٠. BASF developed a commercially feasible manufacturing process that was in use by ١٨٩٧. The annual production of synthetic indigo is estimated at ١٧،٠٠٠ tons world wide (http:// www.)

٢:٣:٣:٢ Natural dyes

Natural dyes are obtained from animal or plant matters without processing. The diversity of natural dye sources is quite vast, superior

plant families carry natural dyes. About ١،١٠٠ plant species can be used as dye plant (Anna and Christian, ٢٠٠٣).

Yellow, red, brown, green and dark grey shades can be obtained from various source of plant materials, these are the most popular colors. Despite the various dyes, the need for indigo as a blue component is persistent (Bechtold, *et al.*, ٢٠٠٢).

Piccagal and Venturi (١٩٩٨) mentioned that plant colorants are used in coloring foods, textiles, cosmetics and pharmaceutical preparations. According to survey conducted in Turkey, Necatt, *et al., (*٢٠٠٤), reported that plants are traditionally used to dye carpets and woven matting in the eastern Mediterranean region, and mentioned that ٣٧ species of plants belonging to ٢٩ families were used in natural dye production.

In traditional dyeing, different materials, such as mud and ash were used with the plant material in order to obtain light and dark shades of color, for example, *Datisca cannabina* gave a bright yellow color, but when mud was added during the dyeing, it gave brown, greenish brown or khaki color (Necatt, *et al.,* ٢٠٠٤).

There is a common misconception that natural dyes are inferior in quality to synthetic dyes, giving only pale color, rather than strong, bright color. In fact, when nylon was first developed, it was necessary to use natural dyes to obtain the best shades demanded by the consumers, as no synthetic alternatives were available. The earliest dye textiles, preserved to the present day, used natural dyes from plants such as woad, madder, indigo, their colors are still recognizable and some vibrant, after many years (Shewry, *et al*., ١٩٩٧).

٢:٣:٤ Economic importance

From the middle ages, the cultivation of dye plants and the further processing and dyeing became an important economic factor in Europe, e.g., woad, *Isatis tinctoria* in Germany and madder, *Rubia tinctorum* in the Netherlands and Southern areas of France. (Anna and Christian ٢٠٠٣). Since the ١٩٩٠s research institutions in Germany, Italy, England, France, Netherlands, and Austria have been dealing with research for the re-introduction of natural dyes. The reasons for this new scientific interest are based on a growing awareness to find sustainable and non-toxic alternatives to synthetic dye, growing market of natural textiles, and the research for additional, economically viable alternative crops for farmers (Anna and Christian ٢٠٠٣). France textile institute gives about ٨٪ annual increase of the market of the natural dyes. Several national and international projects are being set up to plant different natural plant dyes, aiming to an industrial production of natural dyes (http:// www.). A United Nations Development Program (UNDP), Sub-Program on the Development and Use of Natural Dye in Textile has been established in India to address the problems that have rendered large scale production of textile dyed with natural dyes impractical, it also addresses the widely understood need to use environmentally dyeing technologies. Among the key objectives of the UNDP is the economical production of high-quality natural indigo dye using environmentally agricultural practices without the use of synthetic chemicals (http:// www.).

Several national and international projects are being set up to plant different natural plant dyes. In Europe, there are projects of production, extraction methods, and textile applications, and Link program (http:// www.). In Japan, concern for Awa Ai (Indigo) has increased and efforts are being made to preserve and protect this cultural treasure (http:// www.). In Bangladesh, there is a bright prospect for cultivation of natural indigo; cultivation of indigo plants under a project titled (Nilkamol), the interest is growing among farmers, because its cultivation is easy and cheap. Several non – government organizations have undertaken projects to produce high quality indigo, the demand is now increasing (http:// www.).

The need for natural dyes is being shown through several non-alimentary activities, such as

- Art products, decoration, and cosmetic products.
- Printing and computer inks.
- Automobile paints.
- Leather and textiles.

Recently, changes in legislation on the use of some toxic synthetic dye and an increased preference by consumers for natural product have combined to re-awaken an interest in dyes derived from plants, with the limited demand being met by small scale market, resulted in high prices of dye (Shewry, *et al.,* ١٩٩٧).

Piccaglia and Venture (١٩٩٨) suggested that to promote the marketing of the natural dyes, it is necessary to develop the cultivation of the dye plants, extraction techniques and analytical methods for

separating, identifying and quantifying the components of the different classes of pigments.

The total world market for dyestuffs is ٨٠٠ kt, of which, indigo represent ٨٠ kt, and the European market represent approximately ١٠٪ of this (Kerry, *et al.,* ١٩٩٨). The total market for natural dyes would be $ two billion, the European market, estimated at about ١٠٪ of the world market, and the market is expected to grow to possible total of $ ٦٠٠ million. (Shewry, *et al.,* ١٩٩٧).

٢:٤ Plant dyes

٢:٤:١ Lawsone

Lawsone is a color from henna (*Lawsonia alba* and *L. inermis*) used frequently in hair care. The dye is present in the leaves, and is responsible for the red color. This color has been known nearly for ٥٠٠٠ years, and was used by the ancient Egyptians for dying their hair and nails. The leaves are ground into a paste that has a great affinity for protein. Henna is quite innocuous and little allergy have been reported on its use. Lawsone is known to be antibacterial, and has been used to treat skin infection (Dweck, ٢٠٠٢, http://www.).

٢:٤:٢ Lycopene

An extract from tomatoes that gives a red to orange color, and is also reported in other plant sources such as *Calendula officinalis* (Marigold) and *Citrullus lanatus* (Watermelon) (Dweck, ٢٠٠٢).

٢:٤:٣ Madder

Madder is obtained from the plant *Rubia tinctroria*, the generic name means red. It is native to the Mediterranean and Near East, and was widely grown as a dye plant. One form of this dye is sometimes called Turkey red. The plant produced several anthraquinones in its roots. The ٢-٣ years old root of the plants is used. The two red chemicals derived from roots and tubers, are known as alizair and purpurin and usually used with an aluminum mordant.

Changing the mordant can give red, pink, lilac, orange, black and brown colors (Dweck, ٢٠٠٢).

٢:٤:٤ Apigenin

This flavonoid, which occurs widely in plants gives a dull golden yellow color, and is obtained from German chamomile. Apigenin and lutolin are more active than the other flavonoids. The spasomolytic activity of chamomile was attributed to apigenin. It is also found in marigold, *Artemisia inculta*, *Cuminum cyminum*, *Daucus carota*, *Agrimonia eupatoria,* all of which have demonstrated anti-inflammatory activity when used under the right conditions (Dweck, ٢٠٠٢).

٢:٤:٥ Capsanthin and capsorubin

Capsanthin and the related capsorubin are most commonly found in Paprika or *Capsicum annum*. As well as being a dyestuff, it is also used in cosmetics, in ointments, oils and emulsions for its stimulating effect and as a massage (Dweck, ٢٠٠٢).

٢:٤:٦ Carotenes

This is a group of yellow/orange colors extracted from carrots and palm oil. The carotenoids-apart from the chlorophylls, are the largest group of oil soluble pigments found in nature. They are responsible for the yellow color of flowers and pigments of many fruits like paprika, tomato. Carotene is one of the major yellow colors used in food industry and the largest used in dairy and is one of the popular free radical scavengers and antioxidants (Dweck, ٢٠٠٢).

٢:٤:٧ Carthamin

It is found in the flowers of safflower, *Carthamus tinctoria*. Carthamin was used for thousands of years in India and other parts of the Near East. It yields the pigment Carthamin which is yellow-orange in color. A part from the seeds for oil and the flowers for dyeing wool, silk and leather; it was also used by the Indians to dye their official red tape used on legal documents (Dweck, ٢٠٠٢).

٢:٤:٨ Crocin

A bright yellow color that has been used for over thousand years extracted from the fruit of *Gardenia jasminoides*. Generally used as a natural yellow pigment, and also used for irritation, sore and swollen eyes (Dweck, ٢٠٠٢).

٢:٤:٩ Crocetin

It is also known as natural yellow color, saffron, and the dried stigma and tops of the *Crocus sativus* contain crocines, crocetins and picrocrocine. They are delicate colors and should be protected from light. Saffron is used as a food and cosmetic dye and flavoring agent (Dweck, ٢٠٠٢).

٢:٤:١٠ Chlorophyll

Extracted from grasses and alfalfa, it is present in all green plants and has always been a part of man's diet. It is also found in green vegetable such as spinach and the common *Urtica dioica* (Dweck, ٢٠٠٢).

٢:٥ Indigo dye

The word indigo is derived from Greek Indikon and the Latin Indicum, meaning a substance from India, although came from nila, meaning dark blue and survives in the word aniline (http:// www.). The blue dye has been known and used throughout the world for many centuries. It is the natural product of a wide range of plants of different species, genera and even families (Martin-Leake, ١٩٧٥).

Simon, *et al.*, (١٩٨٤) reported that the blue dye was produced by fermentation of the leaves usually with caustic soda or sodium hydrosulfite, and the exudates processed into dry cake. On the other hand, Angelini, *et al.*, (٢٠٠٤) mentioned that the leaves of *P. tinctorium* accumulated large amounts of a colorless glycoside, indicana indoxyle beta glycoside from which the blue dye indigo was synthesized.

٢:٥:١ Source and History

Indigo is among the oldest dyes to be used for textiles and printing.

A variety of plants have provided indigo throughout history, but most natural indigo is obtained from the genus *Indigofera*. To these sources is to add the commercial product, woad, *Isatis tinctoria* and *Polygonum tinctorium*, plants which were formerly much cultivated in France and Germany. (Harvey and John ١٨٩٨).

Indigo blue does not exist as such in the plants. It is developed by the decomposition of the bitter glycoside indican ($C_{٢٦}$ $H_{٣١}$ $NO_{١٧}$) a colorless chromogene, when acted upon by diluted acids or ferments, it absorbed water and hydrolyzed to indigo blue ($C_{١٦}$ $H_{١٠}$. $N_{٢}$ $O_{٢}$) (Harvery and John ١٨٩٨). On the other hand, Minami (٢٠٠١), reported that *Polygonum tinctorium* accumulates large amount of colorless glycoside indican, in the cells of its leaves, and when the leaves are ground, indoxyl is formed from the indican by the action of indican – degrading enzyme, and then oxidized by air forming the blue indigo dye.

٢:٥:٢ Indigo properties

Commercial indigo occurs in hard, porous, brittle lumps or cubes of a dark-blue color, and devoid of taste and odour. Indigo which is firm, dense, not easily broken, and which has a dull or greenish or grayish hue. Its specific gravity varies between ١,٣٢ and ١,٤٥. Commercial indigo contains from ٢٠ to ٨٠٪ of indigo-blue, the remainder consists of indigo - brown, indigo-red, indigo- gluten, water and varying quantities of mineral matters. The ash is not more than ٨٪. Indigo is not affected by

the ordinary solvents, such as water, alcohol, ether, diluted acids and alkalis. However, it can be crystallized from aniline. It is soluble in chloroform, glacial acetic acid, paraffine, castor oil, nitro benzene phenol. Concentrated, especially fuming sulfuric acid readily dissolves indigo, forming deep – blue solution (Harvery and John ١٨٩٨).

٢:٥:٣ Economic importance

Indigo is the most important blue component in the class of natural dyes for cellulose, and protein (Bechtold, *et al.*, ٢٠٠٢). The indigo dye is used for dyeing fabrics especially denim cloth for jeans, printing cotton, and wool.

Popularity and economic value of the dye reached a peak during the Middle Ages, when indigo was the most important dye plant for the blue color in the western portion of the world (Simon, *et al*., ١٩٨٤).

Before the manufacture of synthetic indigo at the end of the nineteen century, natural indigo was probably the most widely used in the textile industry; however the market of natural indigo fell to ٤٪ by ١٩١٤. Recently, resurgence of interest in natural indigo has been shown in the West European and North American market; however, this has not resulted in any clear upturn in international trade. (FAO, ١٩٩٥).

Synthetic Indigo dye production reached more than ٣٣،٠٠٠ tons, mainly used to dye woolen clothes for marine blue textiles with excellent fastness (Bechtold, *et al.*, ٢٠٠٢).

Currently ٨،٠٠٠ tons of indigo are imported to Europe, ٤٠٠ tons of which are natural indigo mainly from India. That share is expected to

triple in the next ten years and new markets are being sought all the time. Inkjet printers, for example, could use indigo (http:// www.).

The current market price is £٣٠ $kg^{-١}$ for natural indigo. The estimate size of the European market for indigo would be £١٢ million and is expected to grow to possible total of £ ٣٦ million. At present, small amount of natural indigo are produced in tropical and subtropical countries, which satisfy the demands of small scale craft dyers (Kerry, *et al.*, ١٩٩٨).

It is also reported that the European indigo market, which is supplied by the synthetic indigo, amounts to ٨،٠٠٠ tons per year. The price of natural indigo varies greatly, about benchmark price of ٨٠ Euro per kg, the market is worth to ٦٤٠ m Euros per year. The target market share of ٥٪ would have an estimated annual value of at least ٣٢ m Euros per year (http:// www.).

٢:٥:٤ Uses

Indigo is rarely used in pharmacy except as test. Dissolved in strong sulfuric acid, it forms indigotin-disulphonic acid, which after suitable treatment is sold in the form of a paste as indigo extract. The sodium salt of indigotin-disulphonic acid is used under the name 'indigo carmine' as a staining agent in microscopy. The sodium salt of orthoitropheny propiolic acid (Nitropropiol) is used as a test of sugar in urine, in the presence of glucose indigo blue is formed by reduction on boiling (http:// www.).

٢:٦ Extraction

The process of extracting indigo dye is quite complicated and involves a lot of labor. The normal commercial process requires cutting the plants and to be tied into bundles and then packed into the fermenting vats and covered with clear fresh water. The plants were allowed to steep till the rapid fermentation, which quickly sets in. The time required being from ١٠ -١٥ hours during which time the indican is hydrolyzed to glucose and indoxyle which passed into aqueous solution. The liquor, which varied from a pale straw color to a golden- yellow, was then run into beaters, where it was agitated either by men or by machinery. The color of the liquid becomes green, and then blue, and finally the indigo separated out as flakes, and precipitated to the bottom of the vats. The indigo is allowed to thoroughly settle, when the supernatant liquid was drawn off (http://www.). The process of extraction of the dye is difficult because of the strong odour that the vat emanates.

In many researches dealing with the production of indigo from natural sources, the amount of indigo formed was determined by analysis of the precursor substances, indicant or indoxyl or by chemical extraction methods (Bechtold, *et al*., ٢٠٠٢). The indigotin and indrubin content in aqueous extract and residues as well as extract with ٩٥٪, ٥٠٪ alcohol were determined by thin layer chromatography (TLC) densitometry. Extraction with alcohol can give more indigotin and indirubin (Zhang, *et al.*, ١٩٩٩).

On the other hand, WU, *et al.*, (١٩٩٩) reported that, *Indigofera tinctoria* was permeabilized with ٢٠٪ methanol at ٢٥ºC released ٨± ٢*µg* indigo g ^¯١ dry plant material excluding roots.

٢:٧ Identification and Quantification

A substance isolated from the leaves of *I. tinctoria* and *I. articulata* was identified as indican, the amount determined by spectrophotometer (Khazhakyan *et al.*, ١٩٨٦). Tetsuok, *et al*., (١٩٩٨) mentioned that two precursors of indigo in the woad plant were quantified by new spectrophotometic method. A method to quantify the indigo precursor indican (indoxyl-beta-D-glucoside) in *Polygonum tinctorium* has been developed; indican was identified and quantified using high performance liquid chromatography (HPLC) coupled to an evaporative light scattering detector (ELSD), with this method it is possible to measure indican content in short time (Angelini, *et al*., ٢٠٠٣). Muagard, *et al*., (٢٠٠١) used HPLC to identify and quantify indigoid pigment (indigo, indirubin, isoindigo and isoindirubin) and indigo precursors. This was confirmed by Anna and Christina (٢٠٠٣), who mentioned that the dyestuff content was analyzed with the HPLC method. Umar Dahot (١٩٩٩) reported that four fractions from the leaves of *Indigofera_oblongifolia* were obtained using Sephadex G-٢٥ column chromatography. A technique for the determination of food dyes using reversed – phase thin layer chromatography on ocatadecyl- modified silica was used by Hisao, *et al*., (١٩٨٧) and found that good separation was obtained between pH ٦,٠-٧,٠. Vandenabeele and Moens (٢٠٠٣) reported that Raman spectroscopy in combination with suitable chemometric methods has the potential to discriminate between natural and synthetic dyes.

CHAPTER THREE

MATERIALS AND METHODS

٣:١ Experimentation

٣-١-١ Experimental Site

The experiments were conducted in the Research Farm of Shambat Research Station that lies at latitude ١٥°,٤٠´ N and longitude ٣٢°,٣٢´ E.

The climate is semi arid with annual rainfall ranging from ١٥٠ to ١٨٠ mm during July to September. The mean temperature and relative humidity during the growing season are given in appendix (١). Shambat soil is heavy clay (٤٣٪ to ٤٨٪ clay) and slightly alkaline with pH ٧,٥-٧,٧ and has electrical conductivity between ٠,٤٢-١,٦ dsm (ElHassan, ١٩٨٩). The mineral chemical composition of Shambat soil is ٠,١٨٪ nitrogen, ٠,٥ppm. phosphorus and ١,٠٣ mg / ١٠٠ potassium (Abdalla, ١٩٨٩).

٣:١:٢ Source of Seeds

The seeds were obtained from wild plants, grown at Kamlien area (Gezira State). The plants were taxonomically identified and classified in the Department of phytochemistry and taxonomy, by Dr. Gamal El Ghazali and herbarium specimens were deposited at the Medicinal and Aromatic Plants Research Institute.

٣-٢ Experiment One

The effect of the growing season on the performance of indigo plant

٣:٢:١ Growing seasons

In the winter season the seeds were sown on the first week of December ٢٠٠٠, first week and last week of January ٢٠٠١.

In the summer season, the first planting was on the first week of April, the second on the first week of May and the third planting on the first week of June ٢٠٠١.

In the autumn season, the first planting was on the first week of July, the second on the first week of August and the third planting on the first week of September ٢٠٠١.

The design used was a randomized complete block design with three replicates.

The experimental site was prepared by "ploughing-discing-harrowing, and leveling". The plot was ridged at ٧٠ cm spacing between ridges and divided into equal plots. The plot size was ٣X٧ m².

Seeds were directly sown according to the specified sowing dates at the rate of ٣-٥ seeds per hole, at spacing of ٧٥ cm between plants. The plants were thinned to one plant per hole after ١٥ days from planting. The plots were irrigated immediately after sowing and irrigation was kept at intervals of ٧-١٠ days thereafter. Hand weeding, was carried out whenever needed.

The first cut was taken after two months from planting, and another two successive cuttings were made at two months interval. The plants were cut with a sickle about ٢-٣ inches above soil level, collected and spread to dry on plastic sheet under partial shade. The leaves were

manually separated from stems after drying, and the leaves dry weight of each plot were recorded. Samples were taken, grounded, packed in plastic bags and kept in dry store for chemical analysis.

٣:٣ Experiment (٢):

Effect of sowing date and spacing on yield and dye content of indigo plant

Four sowing dates were tested in two growing seasons ٢٠٠١/٢٠٠٢ and ٢٠٠٢/٢٠٠٣. Sowing was carried out monthly commencing in June as an early date up to September as late sowing date. Three plant spacings were used ٧٥cm, ١٠٠ cm or ١٢٠ cm between plants. The treatments were arranged in a randomized complete block design with three replicates.

The experimental site was prepared by "ploughing-discing-harrowing, and leveling". The plot was ridged at ٧٠ cm spacing between ridges and divided into equal plots. The plot size was ٣X٧ m².

The seeds were sown directly at distances of (٧٥،١٠٠ or ١٢٠ cm) between plants in the four sowing dates. The field was immediately irrigated after planting; the second irrigation was applied ٧ days later. The plants were thinned to one plant per hole after ١٥ days from planting. The subsequent irrigations were applied at ٧ to ١٠ days intervals. Hand weeding was carried out when needed.

At the flowering stage, about two months from planting, the first cut was made and three successive cuttings were made at two months intervals during the growing season. The plants were cut with a sickle

about two inches above the soil level. The plants were collected in bundles, spread on plastic sheets and allowed to dry in open air under partial shade for one week. The dry leaves were then manually separated from the stems and cleaned. The dry weight of the leaves from each treatment was recorded. A sample of each treatment was ground, and then packed in plastic bags for chemical analysis.

٣:٤ Experiment (٣)

Response of Indigo plants to water management and cutting frequencies

The experiment was designed for two successive seasons ٢٠٠١/٢٠٠٢ and ٢٠٠٢/٢٠٠٣ to study the effect of three watering intervals (i.e. ٧-١٠ or ١٥ days, referred to as WI (١), WI (٢) and WI (٣) respectively, and the effect of stoppage of watering at ٧-١٠ or ١٥ days before the harvesting time (referred to as WS (١), WS (٢) and WS (٣) respectively, in combination with two cutting treatments (referred to as C.T(١) and C.T (٢). In the first treatment C.T (١), the plants were subjected to two cutting times, after one month from planting and a second cut after two months. In the second treatment C.T (٢), the plants were cut once after two months. Treatments were arranged in a split plot design with three replicates; watering intervals were assigned to the main plot and time of water stoppage and methods of cutting treatment to the sub plots.

The experimental site was prepared by ploughing, disc-harrowing, leveling and then ridging at spacing of ٧٠ cm. The area was divided into equal plots (٣X٧ m); the three water intervals (٧-١٠-١٥days) were assigned to the main plots which were separated from each other by border steps one meter wide. Watering termination and cutting frequencies treatments were arranged in the sub-plots.

In the first season, the seeds were sown directly in June, at the rate of ٣-٥ seeds per hole, spacing between holes was ٧٥ cm. The field was irrigated directly after sowing; seven days later a second irrigation was applied. The plants were thinned to one plant per hole after ١٥ days from sowing. The plots were subjected to the different watering intervals after ١٥ days from sowing. In the second season, the seeds were sown in May and all cultural practices were similar to that of the first season.

According to the combination treatment of watering stoppage and cutting frequencies the plants were cut as follows:

١. Two cuts, one after one month from planting and the second after two months from the first cut.

٢. One cut after two months from planting.

The plants were cut with a sickle about ٢ inches above soil level, collected in bundles and dried on plastic sheets. The dry leaves were separated from the stems, and weighed. A sample from each treatment was grounded and packed for chemical analysis.

٣:٥ Experiment (٤)

Harvesting stage and type of fertilizers on yield and dye content of indigo plant

Field experiment was conducted for two consecutive seasons to answer some questions concerning the optimum time to harvest the indigo plant and the response of the plant to fertilizer application under Sudan conditions.

The experiment was conducted in two successive seasons ٢٠٠١/٢٠٠٢ and ٢٠٠٢/٢٠٠٣.

Indigo plants were harvested at four stages of plant growth:

١. After one month from planting.

٢. Before flowering.

٣. At full flowering.

٤. At fruit set.

Four different treatments of fertilizer were also studied:

١. Control, no fertilizer

٢. Composted chicken manure at the rate of ٢,٥ tons /feddan.

٣. Nitrogen fertilizer in the form of urea "٤٦٪ N," at the rate of ٥٠kg/feddan.

٤. Nitrogen fertilizer in the form of urea at the rate of ١٠٠ kg/fed.

In the two growing seasons, the experimental site was prepared as in the previous experiments. The treatments were arranged in a randomized complete block design with three replicates. The plot size was ٣X٧ m. The seeds were directly sown in mid May, ٣-٥ seeds per

hole. Spacing between plants was ٧٥ cm. The field was directly irrigated after planting; the second irrigation was applied ٧ days later. After ١٥ days from planting, the plants were thinned to one plant per hole. The composted chicken manure was added to the specified plots before planting and the field was irrigated before sowing. Nitrogen fertilizer in the form of urea "٤٦٪ N" at the rate of ٥٠ kg per fed, or ١٠٠ kg/fed was applied after one week from thinning. Irrigation was kept at the interval of ٧-١٠ days; other cultural practices were carried throughout the growing season as needed.

According to the time of harvest, each treatment was cut, and then other three successive cuts were made at the ٤ stages of harvest throughout the growing season. The plants were cut with a sickle, tied in bundles and dried on plastic sheets. The weight of the dried leaves for each treatment was recorded, and samples were grounded, and packed in plastic bags for chemical analysis.

٣-٢ Laboratory Work

٣:٢:١ Chemical analysis

The indigo dye was concentrated mainly in the leaves of the plant. In this work the dye was extracted from leaves and extraction was performed following the procedure described by Shugar, (١٩٨١). Soxhlet extractor was used to extract solutes from solids, using optimized desired solvent, as solvent is vaporized; it is allowed to condense on the solid substance to extract soluble compounds. When the liquid level fills the body of the extractor, it automatically siphons into the recovering flask. This process

continues repeatedly as the solvent in the flask vaporizes and condenses (Fig ٢).

Twenty grams of sample as the leave powder were placed in filter paper and transferred to soxhlet apparatus and plugged with cotton wool. Different solvents were tested to determine the most suitable solvent for the extraction. The solvents used as follow:

١- Ethanol (٨٠٪)

٢- Petroleum ether (b.p ٦٠°)

٣- Petroleum ether and ethanol (٨٠٪) successively

٤- Chloroform

According to the solvent, ٢٥٠ ml at temperature ٦٠°C were added and extraction was commenced, it automatically siphons into the recovering flask, this process continues repeatedly as the solvent in flask vaporizes and condenses, it take about ٨ hours until the extraction completely free of green color. Each sample was carried three times.

The extract was evaporated by using rotatory evaporator, and then placed in clean and weighed Petri-dish and allowed to dry at room temperature. After drying the Petri-dish was weighed and dye content was calculated by the following formula

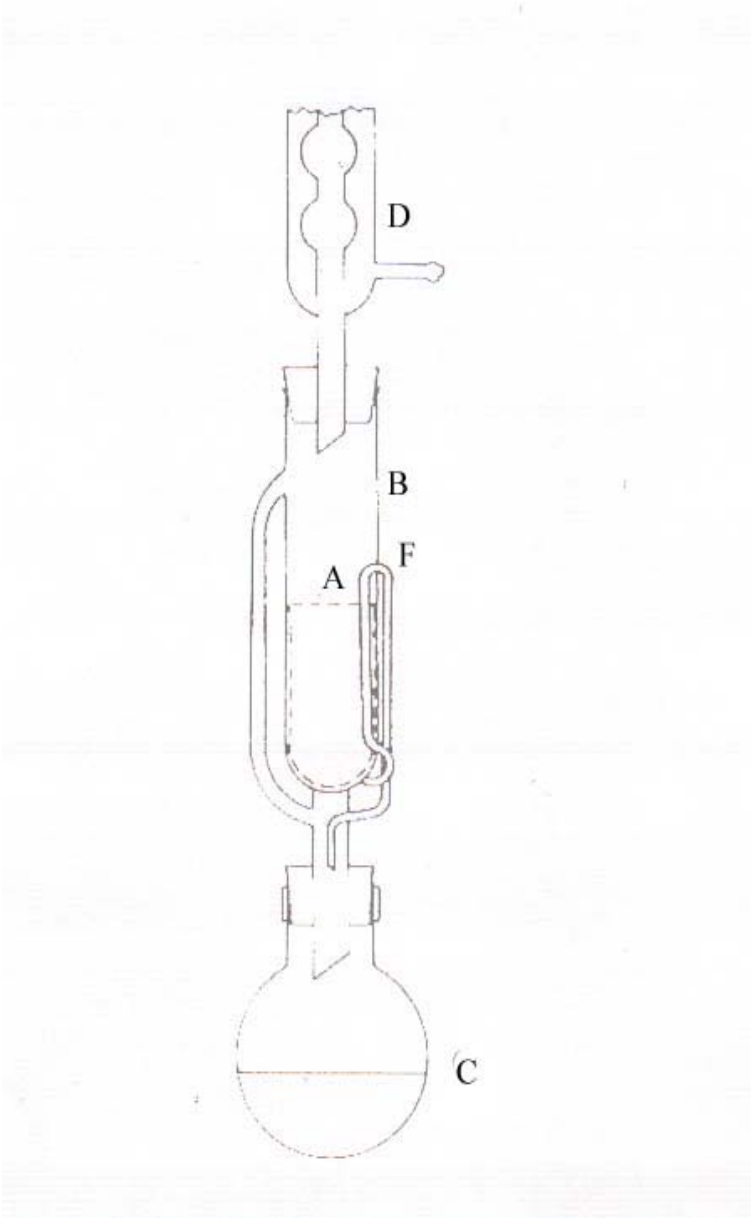
$$\text{Dye content \%} = \frac{W_٢ - W_١}{\text{Weight of sample}} \times ١٠٠$$

Where:-

$W_١$ = weight of Petri-dish

$W_٢$ = weight of Petri dish + dry extract.

Fig (٢) Soxhlet apparatus



A: sample

B: Body of extractor

C: Extracting solvent

D: Re-flux condenser

F: Liquid return siphon

٣:٢:٢ Phytochemical screening

Phytochemical screening was done to determine the chemical constituents of the indigo plant. Chemical groups were carried out according to the procedure described by Harborne (١٩٨٤).

Plant Materials:

Samples of *Indigofera tinctoria* were collected from different localities, Shambat area (Khartoum state), Kamline and abu Ashar (Gezeria state). The samples were authenticated and identified by Dr. Gamal El Ghazali and herbarium specimens were deposited at the Medicinal and Aromatic Plants Research Institute.

The seeds obtained from the wild plants were used in the field experiments. The materials used in Phytochemical screening were obtained from the cultivated plants.

Methods: Preparation of the extract:

١٠ g of the powder plant were refluxed with ١٠٠ ml of ٨٠٪ of ethanol four ٤ hours. The cool solution was filtered and enough ethanol was passed through the volume of the filtrate to ١٠٠ ml. This prepared extract (PE) was used for the various tests.

٣:٢:٢:١ Test for Unsaturated Sterols and Triterpenses:

١٠ ml of the PE was evaporated to dryness on a water path and the cooled residue was stirred several times with petroleum ether to remove most of the coloring materials. The residue was then extracted with ٢٠ ml chloroform. The chloroform solution was dehydrated over sodium sulphate anhydrous. ٥ ml portion of the chloroform solution was mixed with ٠,٥ ml of acetic anhydride followed by ٢ drops of concentrated sulphuric acid.

٣:٢:٢:٢ Test for Alkaloids:

٧,٥ ml of the PE was evaporated to dryness on a water bath. ٥٪ ml of ٢N Hcl was added and stirred while heating on the water bath for ١٠ minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer's reagent was added while to the other tube few drops of Dragenddroff reagent was added.

٣:٢:٢:٣ Test for Flavonoids:

١٧,٥ ml of the PE was evaporated to dryness on water bath, cooled and the residue was defatted by several extractions with petroleum ether and the defatted residue was dissolved in ٣٠ ml of ٨٠٪ ethanol and filtered. The filtrate was used for following tests:

A. ٣ ml of the filtrate in a test tube ١ ml of ١٪ aluminum chloride solution was in methanol was added.

B. ٣ ml of the filtrate in a test tube ١ ml of ١٪ potassium hydroxide solution was added.

C. ٢ ml of the ٠,٥ ml of magnesium were added.

٣:٢:٢:٤ Test for Tannins:

For this test ٧ ml quantity of the PE was evaporated to dryness on water bath. The residue was extracted several times with n-hexance and filtered. The insoluble residue was stirred with ١٠ ml of hot saline solution. The mixture was cooled, filtered and the volume of the filtrate was adjusted to ١٠ ml with more saline solution. ٥ ml of this solution was treated with few drops of gelatin salt reagent. To another portion of this solution, few drops of ferric chloride test reagent were added.

٣:٢:٢:٥ Test for Saponins:

١ g of the original dried powder plant material was placed in a clean test tube. ١٠ ml of distilled water was added and the tube was stopper and vigorously shaken for about ٣٠ seconds. The tube was then allowed to stand and observed for the formation of honeycomb.

٣:٢:٢:٦ Test for Cyanogenic glycoside:

٣ g of the powder plant sample were placed in Erlenmeyer flask and sufficient water was added to moisten the sample, followed by ١ ml of chloroform to enhance every activity. A piece of freshly prepared sodium picrate paper was carefully inserted between a split crocks which was used to stopper the flask.

٣:٢:٢:٧ Test for Anthraquinone glycoside:

١٠ g of the powder plant sample were boiled with ١٠ ml of ٠,٥ N KOH containing ١ml of ٣٪ hydrogen peroxide solution. The mixture was extracted by shaking with ١٠ ml of benzene. ٥ ml of the benzene solution was shaken with ٣ ml of ١٠٪ ammonium hydroxide solution and the two layers were allowed to separate.

٣:٢:٢:٨ Test for Coumarins:

Three grams of the original powdered plant sample boiled with ٢٠ ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of ٠,٥ N KoH put on it. Then the filter paper was inspected under UV light.

٣:٢:٣ Thin layer chromatography

Sample of ethanolic extract of each treatments of samples were used for thin layer chromatography (TLC).

- cultivated plant
- sowing dates x spacing
- wild plant
- fertilizers application x stages of harvest
- Water management
- local market

٣:٢:٣:١ Preparation of silica gel plates

Thirty grams of silica get type GF, placed in ٢٥٠ ml conical flask, mixed with ٦٠ ml distilled water, shacked carefully to form slurry. Then

transferred to a moving Desega spreader, which consists of metal cylinder partly cut out to from a trough. The cylindrical trough was then rotated ١٨٠° to bring slurry into contact with ١٠X٢٠ cm glass plates. The spreader, ٠,٢٥ mm deep was then pushed carefully across a set of glass plates held on spreading board. The plates were activated by heating after they were dry at ١٠٥ºC for about one hour (Stahl, ١٩٦٩).

٣:٢:٣:٢ Solvent systems

Some solvent systems were tested to determine the suitable solvent systems for the separation of dye. The most suitable solvent systems were as followed:

Benzene**:** Petroleum ether at ratio of ٢٠:١

Benzene: Ethyl acetate at ratio of ٨٠:٢٠

٣:٢:٣:٣ Application of test materials:

Ethanolic extract was spotted by a capillary tube, the plates were placed in the tank which contains the solvent system, and after the solvent had moved a distance of ١٥ cm they were removed from the tank and allowed to dry.

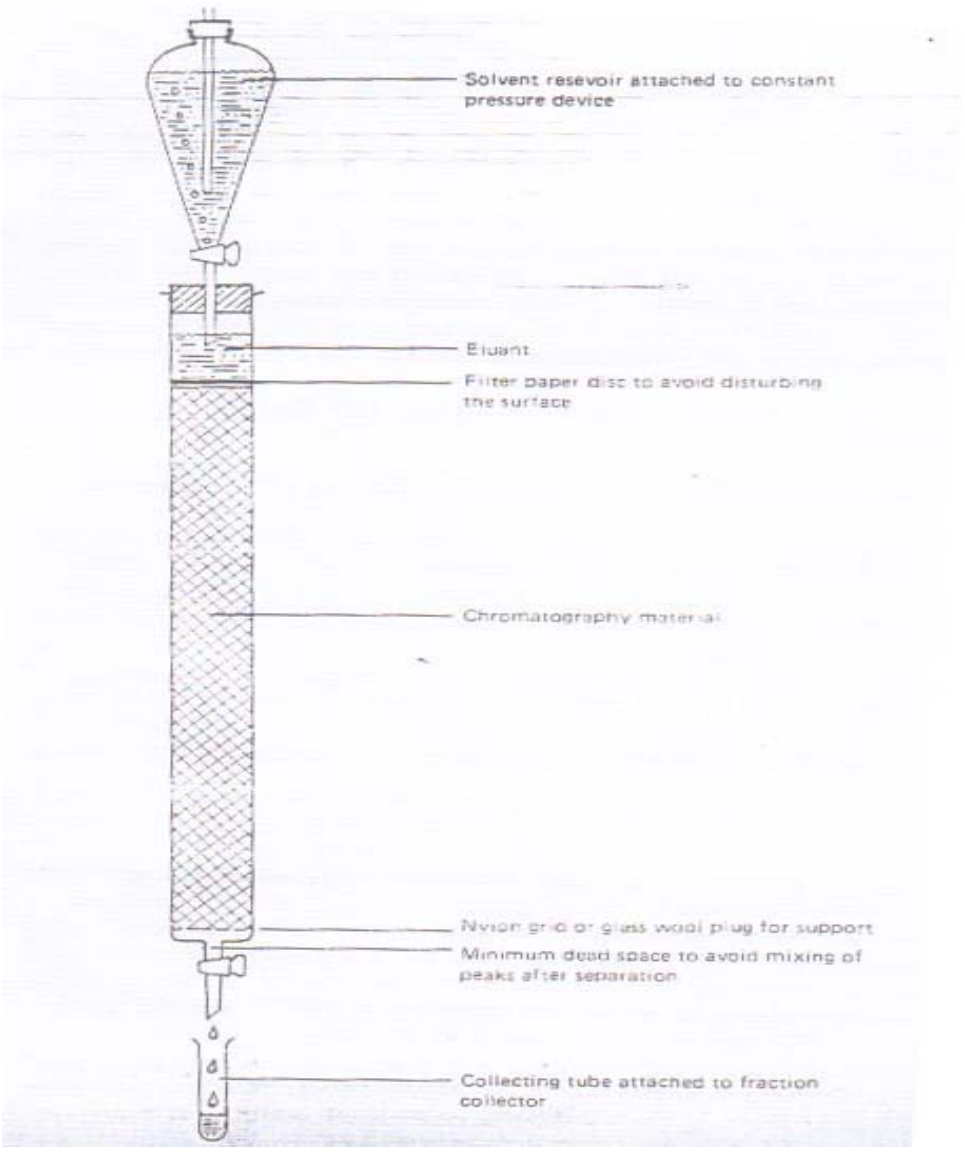
٣:٢:٣: ٤ Detection

Some reagents were examined to determine the most suitable reagent to detect the dye. It was clear that the reagent Aisaldehyde- sulphuric acid was the best reagent. TLC plate was sprayed with about ١٠ ml, heated at ١٠٠ °C for about ٥ minute. The R_f were recorded.

٣:٢:٤ Column chromatography

Separation of compounds by column chromatography was used widely in biochemical work. Columns chromatography are usually glass and generally, long columns give good resolution of components and large columns are better for dealing with large quantities of material (Fig ٣).

Fig (٣) Apparatus of column chromatography


Solvent resevoir attached to constant pressure device
Eluant
Filter paper disc to avoid disturbing the surface
Chromatography material
Nylon grid or glass wool plug for support
Minimum dead space to avoid mixing of peaks after separation
Collecting tube attached to fraction collector

٣:٢:٤:١ Preparation of materials

The powdered leaves (١٠٠g) were extracted in soxhlet with ethanol (٨٠٪). The concentrated residue (٢٠ g) was dissolved in ٢٠ ml methanol the extract adsorbed into silica gel column and concentrated in water bath.

٣:٢:٤:٢ Packing of the column

The chromatography column was packed with material by filling it about one third full with petroleum ether (b.p. ٦٠°C) and slowly adding slurry of the material in the solvent. This was carefully poured down by glass rod to stop air bubbles being trapped in the column. The suspension was allowed to settle and excess solvent run off. After thoroughly washing the column with solvent, cotton wool was placed on top of the adsorbent to avoid disturbing the surface.

٣:٢:٤:٣ Elution and collections

The column was eluted with petroleum ether (b.p. ٦٠°C), followed by petroleum ether\ ethyl acetate by increasing amount of ethyl acetate and finally pure ethyl acetate. The effluent from the column was collected into series of test tubes and concentrated by using rotatory evaporators and then chromatographed by using TLC. The fractions were collected as follows:

١:١ ٣١ test tubes (١٠ml) petroleum ether

Petroleum ether: ethyl acetate

١:٢ ٩٥ : ٥ (١- ٤٧ tubes)

١:٣	٩٢	:	٨	(١- ٧ tubes)
١:٤	٩٠	:	١٠	(١-٥ tubes)
١:٥	٨٨	:	١٢	(١- ١٠ tubes)
١:٦	٨٧	:	١٣	(١-٥ tubes)
١:٧:١	٨٥	:	١٥	(١-٩ tubes)
١:٧:٢	٨٥	:	١٥	(١٠-٢٠ tubes)
١:٨:١	٥٠	:	٥٠	(١-٥ tubes)
١:٨:٢	٥٠	:	٥٠	(٦-١٢ tubes)
١:٩	٤٠	:	٦٠	(١-٥ tubes)
١:١٠	٣٠	:	٧٠	(١-٥ tubes)
١:١١	٢٠	:	٨٠	(١-٢ tubes)
١:١٢	ethyl acetate			(١-٥ tubes)

The elute of the column was monitored by TLC

٣:٢:٤:٤ Preparative thin layer chromatography

Silica gel, ٠،٥mm thickness was used. Fractions ١:٧ and ١:٨ were applied in form of streak and the chromatogram was developed using Benzene**:** Petroleum ether at ratio of ٢٠:١ as solvent system.

CHAPTER FOUR
RESULTS AND DISCUSSION

٤:١ Experiment One

The effect of the growing season on the performance of indigo plant

Initially the experiment was designed for three seasons. However, in the winter season the seeds sown in December ٢٠٠٠, first week and last week of January ٢٠٠١ failed to germinate and the germination percentage was very low and for this reason the result of this season was excluded.

The performance of the plants was measured in terms of leaves dry weight and dye content.

In the summer season, the results presented in table (١) showed that there were highly significant differences (P=٠,٠١) between the first planting in April, second planting in May and third planting in June. In the first cut, the highest yield was obtained from the third planting on the first week of June followed by the second planting on the first week of May, and the lowest yield was obtained from the first planting on the first week of April (Fig ٤).

In the second cut, there were no significant differences between second and the third plantings, and the lowest yield was obtained from first planting (Fig ٤).

In the third cut the highest yield was obtained from the third planting, however there were no significant differences between first and second plantings (Fig ٤).

The highest total yield was obtained from the third planting in June followed by the second planting in May, and the lowest total yield was obtained from the first planting in April (Table ١). The third planting on the first week of June out-yielded the first planting in April and second planting in May by ٥٠,٢٪ and ٣١,١ %, respectively.

In autumn season, there were highly significant differences (P=٠,٠١) between first planting on July, second planting in August and third planting in September. In the first and second cuts, the highest yield was obtained from the first planting in July. The lowest yield was obtained from the third planting in September. In the third cut, the highest yield was obtained from the first planting in July and there were no significant differences between the second planting in August and the third planting in September (Fig ٥).

The highest total yield was obtained from the first planting which out-yielded the second planting in August by ٣٠,٣ % and the third planting in September by ٤٦,٤ % (Table ١).

In both seasons the performance of the plants with respect to the dye content was similar to that of the yield. The main effect of the seasons on dye content was highly significant (P=٠,٠١) (Table ٢).

In the summer season, in the first and the second cut, the third planting in June produced the highest dye content, and the differences were not significant between the first and second plantings. In the third cut, there were no significant differences between the first, second and third plantings (Fig ٦).

Table (١) Effect of planting time on total yield of Indigo plant (Kg/ha)

Planting time	Summer Season	Autumn Season
First planting (April)	٤٢٢١,٦	٦١٤٦,٦
Second planting (May)	٥٠١١,٥	٤٢٨٥,٤
Third planting (June)	٦٥١٤,٩	٣٢٩٧,١
LSD	٢٩٠,٨	٦٥٣,٧
SE ±	٧٤,١	١٦٦,٥

Table (٢) Effect of planting time on total dye content of Indigo plant (%)

Planting time	Summer Season	Autumn Season
First planting (July)	٤١,٨	٤٩,٣
Second planting (August)	٤٩,٧	٤٢,٧
Third planting (September)	٥٠,٥	٣٦,٧
LSD	٤,١	٥,٧
SE ±	١,٠٥	١,٤٦

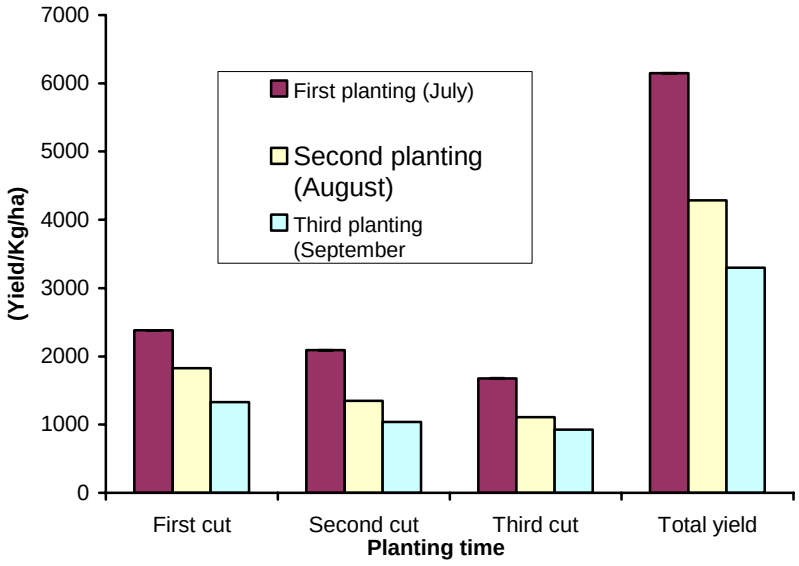


Fig (4) Effect of planting time on yield of indigo plant (Kg/ha)

Fig (5) Effect of planting time on yield of indigo plant (Kg/ha)

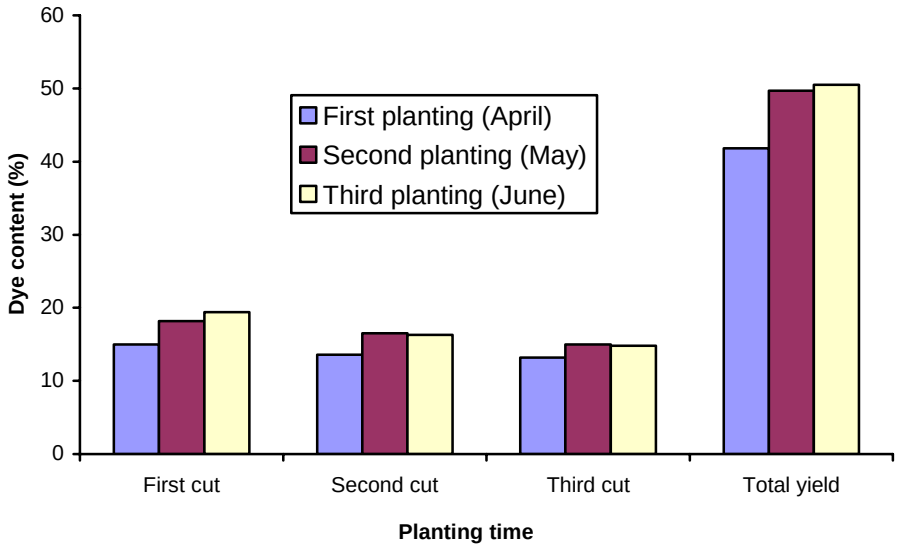


Fig (6) Effect of planting time on dye content of indigo plant (%)

summer season

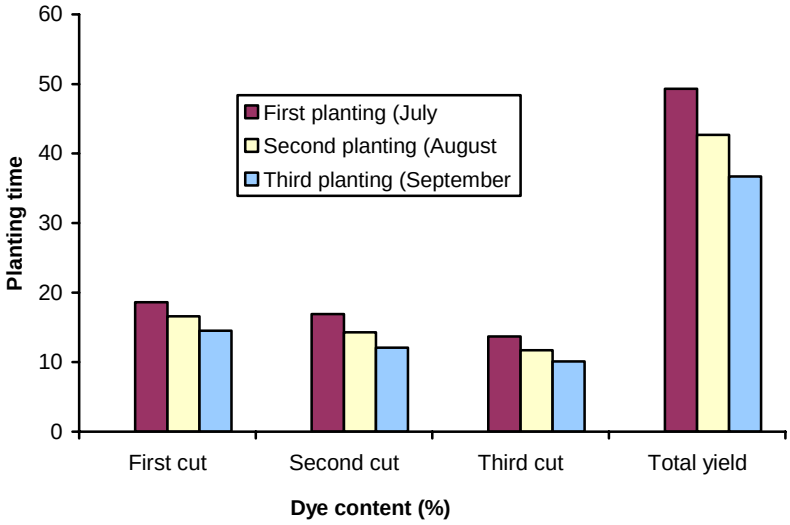


Fig (7) Effect of planting time on dye content of indigo plant (%)

Autumn Season

In the autumn season, in the first cut there were significant differences between the first planting in July and second planting in

August, and the lowest dye content was obtained from the third planting in September, however, in the second and third cuts, there were significant differences between the three planting dates. The highest dye content was obtained from the first planting in July, followed by second planting in August and third planting in September (Fig ٧).

The combined analysis between the summer season and the autumn season is presented in table (٣). It showed that in the first cut there were no significant differences between the two seasons on the yield. But the yield was highly significantly affected by the interaction between the season and the planting date. The late planting in June, out-yielded the first planting in the autumn season by ١٢٪.

In the second and the third cuts the effect of the season on the performance of the indigo plant was highly significant (P=٠,٠١). The summer season out- yielded autumn in the second cut by ١٨,٣ % and in the third cut by ١٤,٦٪. The total yield obtained in the summer season was more than ١٢,٨٪ of the autumn season (Table ٣).

The results presented in table (٤) show that the season has no significant effect on dye content in the first and second cut. However, in the third cut, the dye content was highly significant affected by the season, and that the dye obtained from the summer season was more than ١٧,٤ % of the autumn season.

The total dye content obtained from the summer season out-yielded the dye content obtained from the autumn season by ٩,٥٪.

Table (٣) Mean of yield (Kg/ha) obtained for two seasons

Season	First cut	Second cut	Third cut	Total yield
Summer	١٩٧٢,٥	١٨٢٧,١	١٤٥٠,٠	٥٢٤٩,٣
Autumn	١٨٤٥,٥	١٤٩٣,٠	١٢٣٧,٨	٤٥٧٦,٣
significant	NS	*	**	*

NS= Not significant
* = significant at ٠,٠٥٪
** = significant at ٠,٠١٪

Table (٤) Mean of dye content (%) obtained for two seasons

Season	First cut	Second cut	Third cut	Total yield
Summer	١٧,٥	١٥,٥	١٤,٣	٤٧,٣
Autumn	١٦,٦	١٤,٤	١١,٨	٤٢,٨
significant	NS	*	**	*

NS= Not significant
* = significant at ٠,٠٥٪
** = significant at ٠,٠١٪

Indigo plant performance in the different seasons indicated that the highest yield and dye content were associated with the summer season. This could be attributed to the different temperature prevailing during growth season. Temperature seems to be the most significant environmental variable enhancing plant growth, planting in May and June received warmer temperatures especially during the early stages of growth. In the summer season the first and second cuts were taken in autumn season which attributed to the favorable conditions for flowering from July to September. In the autumn season the second and third cuts were taken in the winter season and resulted in lower yield and dye content. This result may be attributed to the low temperature which seems to be unfavorable for growing. The results are consistent with those of Brian, *et al*., (١٩٩٧) who reported that *Indigofera jucanda* in South Africa is grown in summer with moderate to high rainfall and Angelini, *et al.,* (٢٠٠٤) who reported that under rainfall conditions in Italy, very high indican content and a potential high indigo yield can be obtained by cultivation of the dye plant, *Polygonum tinctorium*.

The same trend was reported for cowpea, the most important leguminous fodder crop, that suitable growth and yield was obtained in both summer and rainy season (http://www.). Failure of crop planted in winter season may be attributed to the low temperature (about ١٥°C) which is unfavorable for seeds germination. This result was confirmed by another web site (http://www.) it was reported that the young plant

became pale yellow and may die if the temperature was low for even one day.

٤:٢ Experiment (٢):

Effect of sowing date and spacing on yield and dye content of indigo plant

The experiment was carried out under field conditions of Shambat to determine the effect of sowing dates and plant spacings on yield and dye content. Differences in yield for the four cuts and for the total yield were statistically highly significant (P=٠,٠١) among sowing dates in the two seasons. Fig (٨) showed that the first sowing in June produced the highest yield, significantly out-yielding late sowing in September by ٤٥,٥ % in the first cut, ٥٠,٠ % in the second, ٣٩,١٪ in the third and ٢٨,٩ %, in the fourth cut. The total yield obtained from June sowing out yielded the late sowing in September by ٤٢,٢ % (Table ٥).

Fig (٩) shows the results of the second season. The first sowing in June out-yielded the late sowing in September by ٣٧,٧ % in first cut, ٤٣,٥٪ in second, ٣٥,٣ % in third and ٢٣,١ % in the fourth cut.

The highest total yield, in the second season was obtained by planting in June which out-yielded the late sowing in September by ٣٥,٥ % (Table ٦)

Highly significant differences (P=٠,٠٠١) were noted in the dye content between the different sowing dates in both seasons. In the first

season (Fig ١٠) the sowing in June resulted in the highest dye content in the four cuts while the lowest dye content was obtained from the late sowing in September in the four cuts. The same trend was obtained in the second season (Fig ١١). Statistical differences in the total dye content between the different sowing dates were noted in

Table (٥) Effect of sowing dates and plant spacing on the total yield

First season ٢٠٠١/ ٢٠٠٢

Yield/Kg/ha

Sowing dates	Spacing ٧٥ cm	Spacing ١٠٠ cm	Spacing ١٢٠ cm	means
June	٨٧٠٩,٢	٧٤٩٢,٩	٥٨٥٥,٣	٧٣٥٢,٥
July	٧١٢٠,٦	٦١٨١,٠	٥٧٨٥,٠	٦٣٦٢,٢
August	٥٨٣٠,٧	٤٩٨١,٠	٤٤٠٣,٨	٥٠٧١,٨
September	٤٨٤٢,٣	٤١٢٤,٥	٣٧٨٩,٨	٤٢٥٢,٣
means	٦٦٢٥,٦	٥٦٩٤,٩	٤٦٩٤,٩	
LSD	٦٣٤,٨			٧٧٣,٠
SE ±	٤٦,٤			٢٤٩,٩

Table (٦) Effect of sowing dates and plant spacing on the total yield

Second season ٢٠٠٢/٢٠٠٣

Yield/Kg/ha

Sowing dates	Spacing ٧٥ cm	Spacing ١٠٠ cm	Spacing ١٢٠ cm	means
June	٨٨٨٤,٥	٧٣٩٦,٢	٦٥٦٨,٨	٧٦١٦,٥
July	٧٥٤٨,٦	٦١٨٤,٨	٥٢٨٥,٢	٦٣٣٩,٥
August	٦٣٢٢,١	٥٤٣٩,٩	٤٨٠٥,٢	٥٥٢٢,٤
September	٥٧٠٨,٨	٤٨٦٤,٧	٤١٧٤,٥	٤٩١٦,٠
means	٧١١٦,٠	٥٩٧١,٤	٥٢٠٨,٤	
LSD	٣٨١,٧			٤٤٠,٨

SE ±	١٣٠,٢	١٥٠,٣

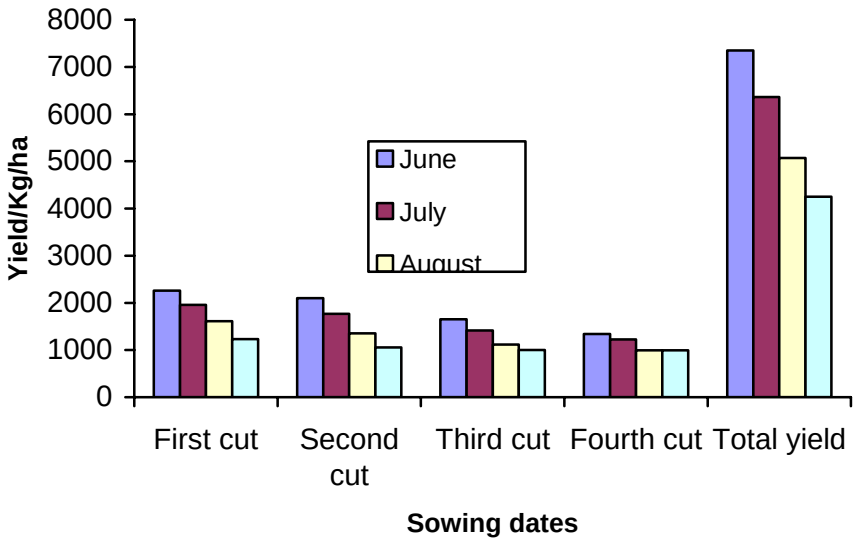


Fig (8) Effect of sowing dates on the yield

(First Season 2001/2002)

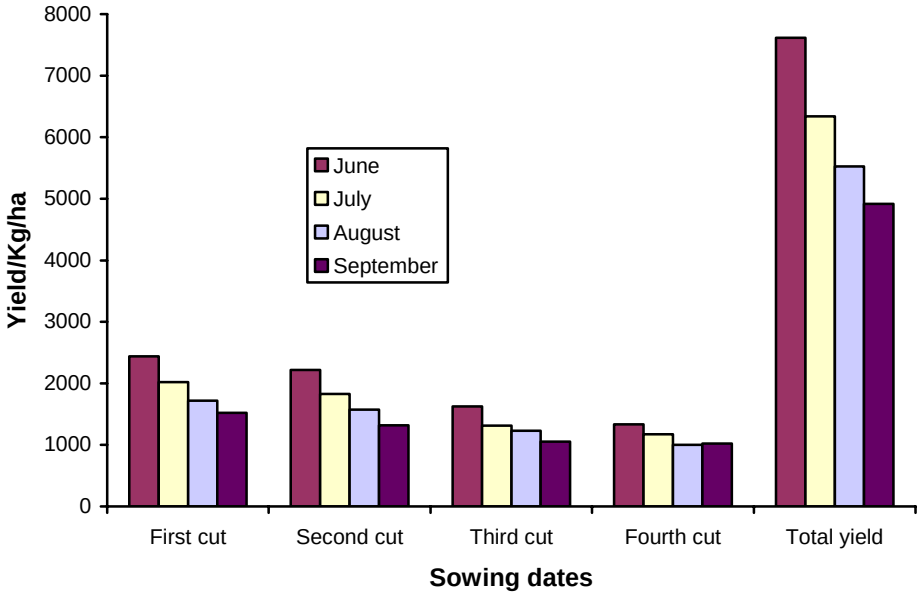


Fig (9) Effect of sowing date on the yield

(Second Season 2002/2003)

Table (٧) Effect of sowing dates and plant spacing on the total dye content (%)

First season ٢٠٠١/ ٢٠٠٢

Sowing dates	Spacing ٧٥ cm	Spacing ١٠٠ cm	Spacing ١٢٥ cm	means

June	٧٢,٧	٦٦,٨	٦١,٣	٦٦,٩
July	٦٢,٨	٦٠,٩	٥٣,٧	٥٩,١
August	٥٦,١	٤٨,٨	٤٤,٧	٤٩,٩
September	٤١,٩	٣٩,١	٣٢,٦	٣٧,٩
means	٥٨,٤	٥٣,٩	٤٨,٠	
LSD				٣,٠٨
SE ±				١,٠٥

Table (٨) Effect of sowing dates and plant spacing on the total dye content (%)

Second season ٢٠٠٢/ ٢٠٠٣

Sowing dates	Spacing ٧٥	Spacing ١٠٠	Spacing	means

	cm	cm	١٢٠ cm	
June	٧٤,٩	٧١,٧	٦٧,٠	٧١,٢
July	٦٨,٧	٦١,٢	٥٤,٩	٦١,٦
August	٥٧,٨	٤٨,١	٤٦,٣	٥٠,٧
September	٤٧,٨	٣٩,٦	٣٥,٠	٤٠,٨
means	٦٢,٣	٥٥,٢	٥٠,٨	٢,٥٧
LSD	٢,٢٣			
SE ±	٠,٧٦			

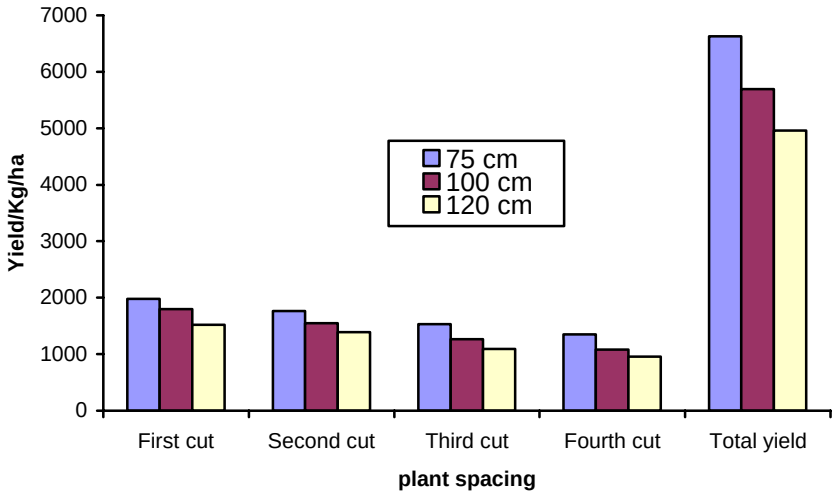


Fig (12) Effect of plant spacing on the yield

(First season 2001/2002)

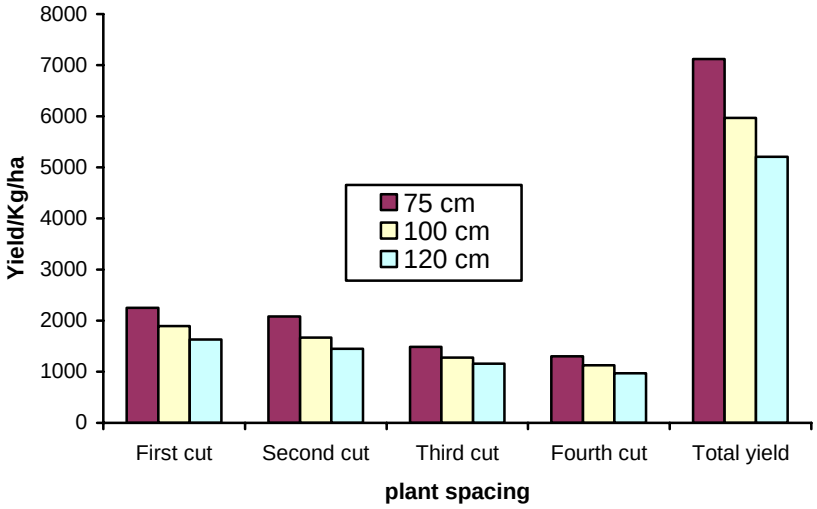


Fig (13) Effect of plant spacing on the yield

(second Season 2002/2003)

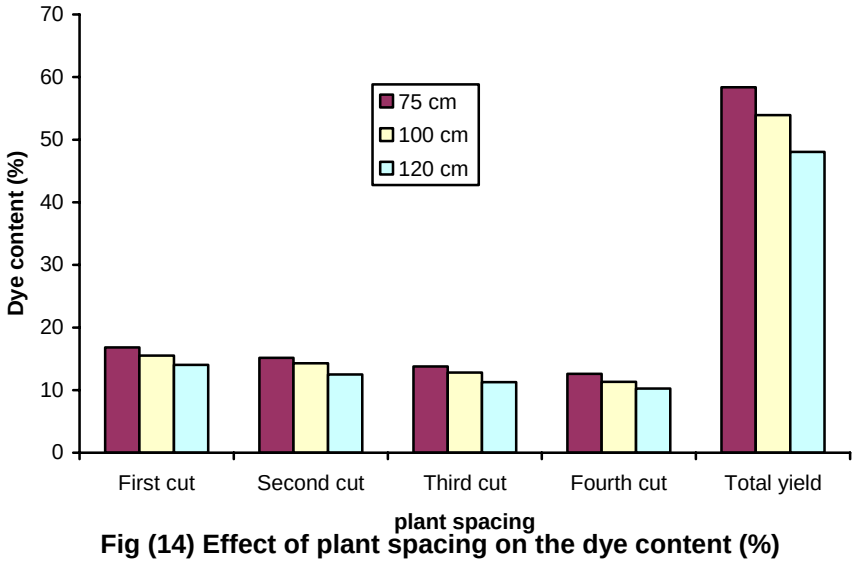


Fig (14) Effect of plant spacing on the dye content (%)

(First Season 2001/2002)

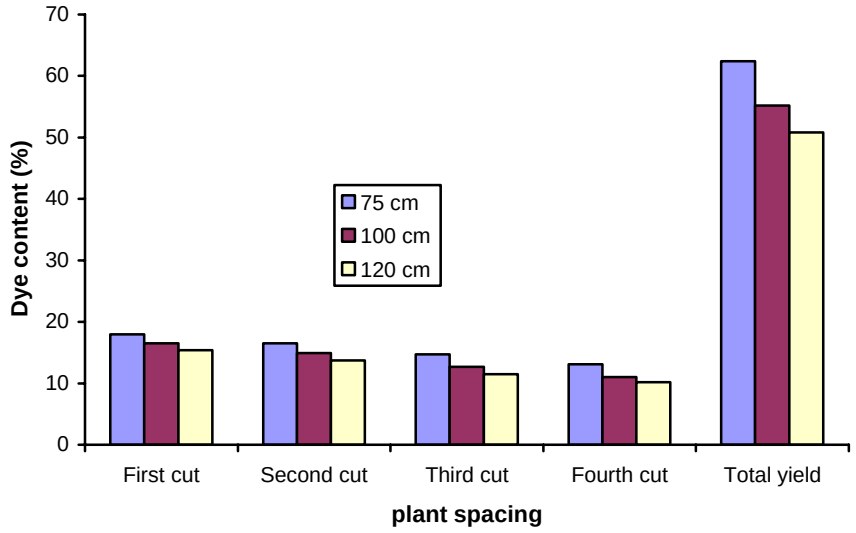


Fig (15) Effect of plant spacing on the dye content (%)

(Second Season 2002/2003)

both seasons (Table ٧ and ٨). The total dye content decreased progressively with delayed sowing.

Plant spacing significantly (P=٠,٠١) affected the yield in the four cuttings in both seasons (Fig ١٢ and ١٣). It was clear that the highest yield was obtained from the closest plant spacing (٧٥ cm), the yield decreased progressively with increasing plant spacing.

Significant differences in the total yield were noted. In the first season (table ٥), closer plant spacing (٧٥ cm) gave ١٤ % and ٢٥ % more total yield than the wide spacing ١٠٠ cm and ١٢٠ cm respectively. Also in the second season planting at ٧٥ cm between plant spacing gave total yield more than ١٦ % and ٢٦,٨ % of the wide spacing, respectively (Table ٦).

Dye content followed a similar trend as yield and was significantly influenced by plant spacing. In both seasons (Fig ١٤ and ١٥) ٧٥ cm spacing between plants gave the highest dye content in the four cuts, and the lowest dye content was obtained from ١٠٠ cm followed by ١٢٠ cm. Total dye content followed a similar trend and was significantly influenced by plant spacing. In both seasons (Table ٧ and ٨) ٧٥ cm spacing between plants gave the highest total dye content.

No significant differences in yield and dye content in the four cuts were noted due to the interactions between sowing dates and plant spacing treatments in both seasons.

There was no available literature on the effect of sowing date and plant spacing on the performance of the indigo plant with regard to the yield or dye content.

Sowing date significantly influenced the yield and dye content in the four cuts and total yield. Moreover, the pattern of influence was similar in both seasons showing decline in the yield and dye content with delayed sowing dates. These differences are attributed to variation in climatic conditions, that is in the first sowing in June the plants were growing in autumn months and the first and second cuts were taken in August and the first week of October. The plants, therefore, had a better chance for efficient utilization of most of the growing conditions and expressed their maximum vegetative growth capacity before the prevalence of the suitable environment. This trend is in general agreement with the findings of Khazhakyan, *et al.*, (١٩٨٦).

Late sowing of the plants took place at lower temperature which might have affected the growth resulting in reduced yield and dye content.

It was evident that closer spacing enables the plant to produce significantly higher yields and dye content. This could be explained on the basis that closer spacing resulted in higher plant populations per unit area. Also in the seedling stage, the plants were closer to each other, creating a macroclimate optimum for their growth and good crop establishment. On the other hand, closer spacing decreased weeds competition. FAO document (١٩٩٥) reported that plant spacing of ٥٠-٦٠ is common. (Ahmed, ١٩٧٨) found that closer spacing between and within rows significantly increased the biological and agricultural yield of cowpea and in lubia closer spacing increased yield but differences did not reach significant level.

٤:٣ Experiment (٣)

Response of Indigo plants to water management and cutting frequencies

The results of water management in both seasons showed that the effect of watering intervals on yield was highly significant (P=٠,٠١) (Fig ١٦). Yield increased with short irrigation intervals; one week irrigation interval resulted in the highest yield; and out yielded the two weeks interval by ٣٩,٦٪ and ٢٧,٣٪ in the first and second season respectively (Tables ٩ and ١٠).

The effect of the irrigation intervals on dye content was highly significant (P=٠,٠١). Dye content obtained from plants irrigated weekly was more than that obtained from the plants irrigated at two weeks interval by ٣٧,٢٪ in the first season and ٣٨,٥٪ in the second season (Fig ١٧). However, in the first season there was no significant difference in dye content between one week and ١٠ days intervals.

Data presented in (tables ٩ and ١٠) indicated that the effect of water stoppage before harvesting on yield was highly significant (P=٠,١). In both seasons, yield decreased by increasing termination of watering interval. When irrigation was stopped one week before flowering, yield was higher than that obtained under longer intervals of water stoppage.

Dye content was significantly influenced by the intervals of water stoppage. The same trend was obtained in both seasons. Stoppage irrigation two weeks before flowering reduced dye content compared with other intervals of water stoppage (Fig ١٧).

Interactions among and between watering intervals and water stoppage intervals in yield were statistically significant in the second season (table ١٠), but, in the first season the interactions among and between these treatments were not significant (Table ٩).

No significant interactions were noted between watering intervals and water stoppage on dye content.

The effects of cutting treatment on yield and dye content were very much pronounced in both seasons (Fig ١٦ and fig ١٧). Plants subjected to two cuts, (one cut after one month from planting and second cut after two months from first cut) out-yielded the plants subjected to one cut after two months from planting by ٢٩,٢٪ and ٢٧,٨ % in the first and second seasons respectively. Both yield and dye content responded similarly to the cutting treatment.

In both seasons two cuttings produced higher dye content compared to one cut, the dye content increased by ٤٦,٣ % and ٥١,٠ % in the first and second season, respectively (Fig ١٧).

The interactions between watering intervals and cutting treatments, on yield and dye content, were highly significant, in the first season (table ١١), in the second season, interactions had no effect on yield (table ١٢); however, effect on dye content was highly significant (tables ١٣ and ١٤).

All the interactions among and between water stoppage and cutting treatments had no significant effect on yield in both seasons, however, interactions on dye content in the first season was significant

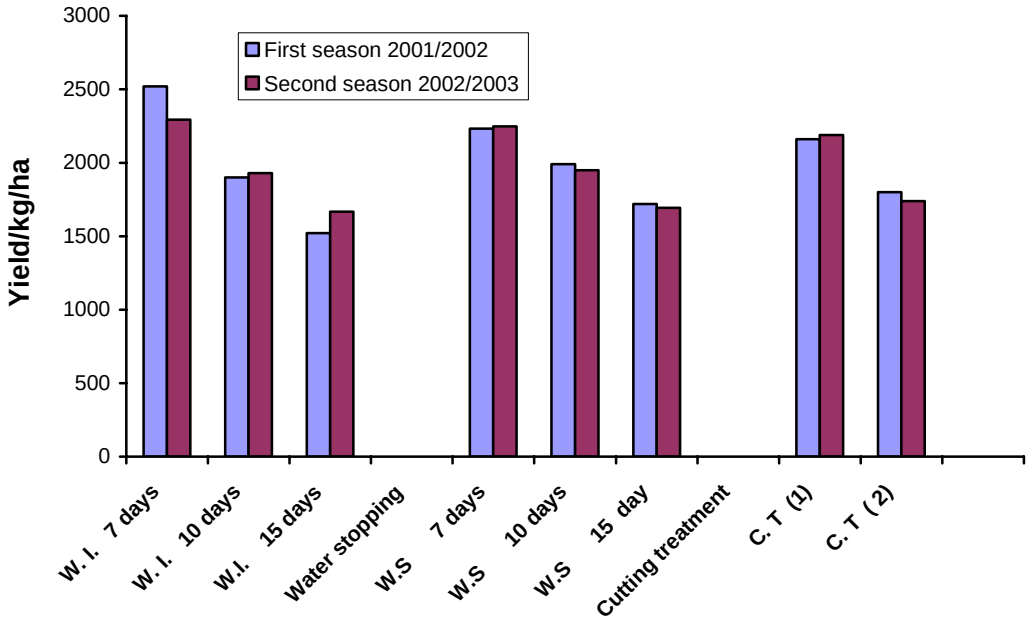


Fig (16) Effect of watering intervals, water stoppage and cutting treatments on yield (kg/ha)

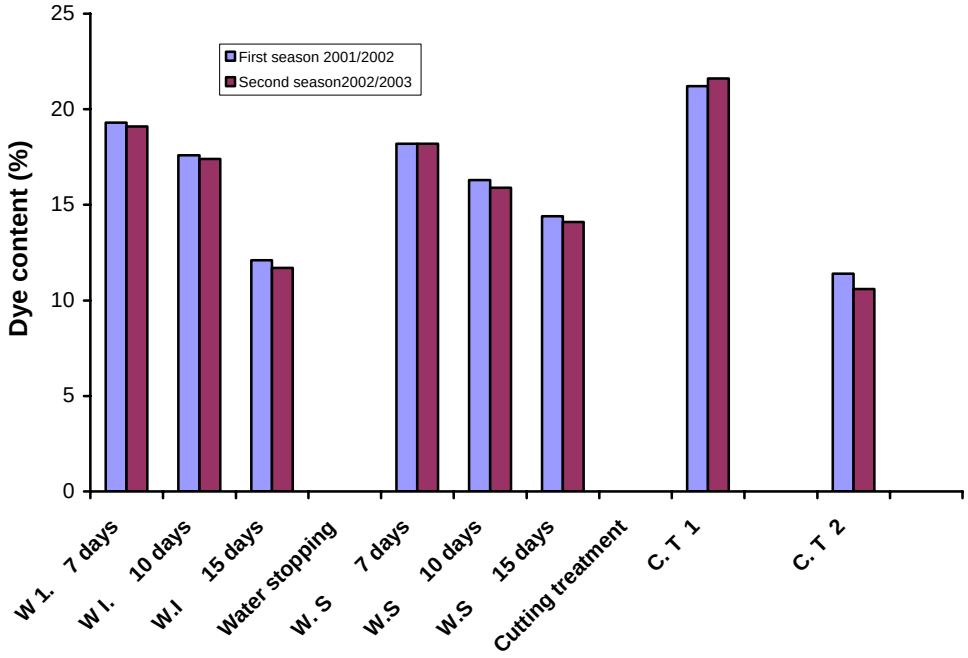


Fig (17) Effect of watering intervals, water stoppage and cutting treatments on dye content (%)

Table (٩) Effect of watering intervals and water stoppage

Yield (kg/ha)

First season ٢٠٠١/٢٠٠٢

Watering intervals	Water stoppage			means
	W.S ٧ days	W.S ١٠ days	W.S ١٥ days	
W.I ٧ days	٢٧٩٦,١	٢٥١٤,٠	٢٢٤٨,٧	٢٥١٩,٦
W.I ١٠ days	٢٢٣٨,٠	١٩٢٥,٨	١٥٣٩,١	١٩٠١,٠

W.I ١٥ days	١٦٦٢,٠	١٥٣,٩	١٣٦٨,٥	١٥٢٠,٨
means	٢٢٣٢,٠	١٩٩٠,٦	١٧١٨,٨	
LSD	٢٠١,٢			
SE ±	٦٩,٧			

Table (١٠) Effect of watering intervals and water stoppage

Yield (kg/ha)

Second season ٢٠٠٢/٢٠٠٣

Watering intervals	Water stoppage			means
	W.S ٧ days	W.S ١٠ days	W.S ١٥ days	
W.I ٧ days	٢٧٠٦,٥	٢٢١٠,٣	١٩٦٣,٩	٢٢٩٣,٦

W.I ١٠ days	٢١٢١,٢	١٨٨٤,٢	١٧٨٨,٣	١٩٣١,٢
W.I ١٥ days	١٩١٦,٧	١٧٥٥,٣	١٣٢٩,٩	١٦٦٧,٣
means	٢٢٤٨,١	١٩٤٩,٩	١٦٩٤,١	
LSD	٢٣٤,٠			
SE ±	٨٠,٩			

Table (١١) Effect of watering intervals and cutting treatments

Yield (kg/ha)

First season ٢٠٠١/٢٠٠٢

watering intervals	cutting treatments		means
	C. T (١)	C. T (٢)	

W.I ٧ day	٢٧٩٣,١	٢٢٤٩,١	٢٥١٩,٦
W.I ١٠ days	٢٠٦٢,٩	١٧٣٩,٠	١٩٠١,٠
W.I ١٥ days	١٦٢٦,٣	١٤١٥,٣	١٥٢٠,٨
means	٢١٦٠,٨	١٨٠٠,١	
LSD	١٦٤,٣		
SE ±	٥٦,٨		

Table (١٢) Effect of watering intervals and cutting treatments

Yield (kg/ha)

Second season ٢٠٠٢/٢٠٠٣

watering intervals	cutting treatments		means
	C. T (١)	C. T (٢)	
W.I ٧ day	٢٦٠٥,٩	١٩٨١,٣	٢٢٩٣,٦
W.I ١٠ days	٢١٢٤,٥	١٧٣٧,٩	١٩٣١,٢
W.I ١٥ days	١٨٣٧,٧	١٤٩٦,٩	١٦٦٧,٣
means	٢١٨٩,٤	١٧٣٨,٧	
LSD	١٩١,٠		
SE ±	٦٦,١		

Table (١٣) Effect of watering intervals and cutting treatments on dye content (%)

First season ٢٠٠١/٢٠٠٢

watering intervals	cutting treatments		means
	C. T (١)	C. T (٢)	
W.I ٧ day	٢٥,١	١٣,٤	١٩,٣
W.I ١٠ days	٢٣,٠	١٢,٢	١٧,٦
W.I ١٥ days	١٥,٥	٨,٧	١٢,١
means	٢١,٢	١١,٤	
LSD	٠,٩٠		
SE ±	٠,٣١		

Table (١٤) Effect of watering intervals and cutting treatments on dye content (%)

Second season ٢٠٠٢/٢٠٠٣

watering intervals	cutting treatments		means
	C. T (١)	C. T (٢)	
W.I ٧ day	٢٦,٠	١٢,٢	١٩,١
W.I ١٠ days	٢٣,٧	١١,٢	١٧,٤
W.I ١٥ days	١٥,١	٨,٤	١١,٧
means	٢١,٦	١٠,٦	
LSD	١,٥٣		
SE ±	٠,٥٣		

Table (١٥) Effect of water stoppage and cutting treatments on dye content (%)

First season ٢٠٠١/٢٠٠٢

Water stoppage	cutting treatments		means
	C. T (١)	C. T (٢)	
W.I ٧ day	٢٣,٧	١٢,٧	١٨,٢
W.I ١٠ days	٢٠,٩	١١,٧	١٦,٣
W.I ١٥ days	١٩,٠	٩,٨	١٤,٤
means	٢١,٢	١١,٤	
LSD	٠,٩٠		
SE ±	٠,٣١		

Table (١٦) Effect of water stoppage and cutting treatments on dye content (%)

Second season ٢٠٠٢/٢٠٠٣

Water stoppage	cutting treatments		means
	C. T (١)	C. T (٢)	
W.I ٧ day	٢٤,١	١٢,٣	١٨,٢
W.I ١٠ days	٢١,٤	١٠,٥	١٥,٩
W.I ١٥ days	١٩,٢	٩,٠	١٤,١
means	٢١,٦	١٠,٦	
LSD	١,٥٣		
SE ±	٠,٥٣		

but in the second season, interactions were not significant (Tables ١٥ and ١٦).

In both seasons effects of the interactions between the three factors studied were not statistically significant neither on yield nor dye content.

No proper research on the effect of water management on the indigo plant was conducted in the Sudan. However, relevant studies on other leguminous crops are available. Increase in yield attributed to shorter watering intervals was reported.

This might be related to the good establishment of the plants under a watering regime of one week interval. Well-watered plants are active and have the ability to explore a large volume of soil and hence have more accessibility to nutrients. Ageeb, (١٩٨١) working on beans found that an increase in watering interval resulted in significant reduction in plant height. Increasing watering intervals decreased yield attributes of faba bean (*Vicia faba* L.) (Elamin, ١٩٨٤). Salih (١٩٩٢) and Abdalla, (٢٠٠٢) found that plant height was reduced by longer irrigation interval. Nabag, (٢٠٠٢) reported that in snap bean, leaf area index and number of branches were reduced by longer irrigation intervals. Ahmed (١٩٨٨) reported that extended irrigation intervals reduced the vegetative and reproductive attributes of cowpea (*Vigna unguiculata*). Abdelrhman (٢٠٠٥) working on Alexandrian senna (*Cassia acutifolia*) reported that irrigation interval of ٧ days significantly increased the vegetative growth over ١٠ days, and ١٤ days intervals. The results obtained are in harmony

with the findings of Mohamed (١٩٩٠) who found that the yield of Berseem, (*Medicago sativa*), under Shambat conditions, increased with decreasing intervals of watering and that cutting frequencies significantly affected the yield.

The results indicated that termination of irrigation ٧ days before flowering resulted in higher yield and higher dye content compared with other treatments. These might be attributed to the fact that water stress reduced vegetative growth by reducing photosynthesis. Prolonged water shortage may lead to irreversible effects including loss of photosynthetic capacity, and shedding of leaves. The effect of soil moisture of various intensities at specific stages of growth of beans was determined by Robins and Domingo (١٩٥٦); the soil moisture treatments consisted of omitting irrigation at three different stages of growth, prior to flowering, during flowering and prior to harvest. They reported that yield was reduced when soil moisture stress occurred at any one of these growth stages. The development of the plants was retarded by moisture deficits before flowering but was hastened during flowering and at late stage of growth. In broad beans, ElNadi, (١٩٦٦) reported that loss in potential yield is likely to be greatest if the plants suffer from water stress during the most critical phase of their growth, and that the number of branches per plant decreased by water stress during the vegetative phase. ElNadi, (١٩٧٠) and Karmanous, (١٩٧٨) mentioned that leaf area index decreased by water stress. These results were confirmed by Elamin, (١٩٨٤) who found that water stress decreased stem extension and consequently plant height and the phase of flowering was more sensitive to drought, resulting in great reduction in attributes of vegetative and reproductive

growth. In cowpea, Suliman (٢٠٠٠) reported that water stress during the reproductive stage hastened flowering and maturity. Effect of drought under different developmental stages on yield and yield components of faba bean had been studied, yield components can be affected by water stress at most of the stages of development, drought stress reduced plant height, number of branches and date of flowering (Mohamed, ٢٠٠٣). These results were in line with those of Ahmed, (٢٠٠٢) who found at Hudieba Research Station that the response of faba bean to water stress by subjecting the crop to stress withholding irrigation during vegetative stage, flowering or pod set stages resulted in great reduction in growth attributes. Adequate moisture is extremely important to snap beans at early stages of plant development due to its influence on stand establishment; beans are most sensitive to moisture stress during flowering; water deficits during this period will have greatest negative impact on yield and quality (Ebraheim, ٢٠٠٣).

On the other hand, Brain *et al*, ١٩٩٧) found that when *Indigofera juncunda* was pruned, the plant responded with vigorous new growth which produced masses of leaves. The effect of cutting frequencies on yield and dye content was associated with new vegetative growth and increased branching and production of more leaves.

Experiment (٤)

Harvesting stage and type of fertilizers on yield and dye content of indigo plant

Analysis of variance showed significant differences in yield and dye content between the different stages of harvest. In both seasons, harvesting at full flowering stage produced the highest yield in the four cuts (Tables ١٧, ١٨).

In the first season, the plants harvested at full flowering stage gave the highest yield in the first and second cuts, followed by the stage before flowering. There were no significant differences between the other two stages of harvest i.e. harvesting after one month from planting and at fruit setting stage. In the third cut, harvesting at full flowering stage gave the highest yield, followed by harvesting before flowering. Harvesting plants after one month from planting gave the lowest yield followed by harvesting at fruit setting. In the fourth cut, there was no significant difference in yield between full flowering and before flowering stages. The lowest yield was obtained from plants harvested after one month from planting followed by harvesting at fruit set (Fig ١٨).

The effect of the harvesting stage on the total yield was highly significant. Harvesting at full flowering stage significantly out-yielded the other stages, i.e. before flowering, at fruit setting, and harvesting after one month from planting by ١٢,٣٥٪, ٢٩,٣٨٪ and ٣٧,٧٥٪ respectively (Table ١٧).

In the second season, the highest yield in the first and second cuts was obtained at full flowering stage (Fig ١٩). Differences between harvesting before flowering and at fruit setting stages were slight. The lowest yield was obtained when plants were harvested after one month from planting. Harvesting at full flowering stage gave higher yield in the third and fourth cuts, followed by harvesting before flowering stage.

There were no significant differences in yield between plants harvested at fruit set and those harvested after one month from planting. On the other hand, the total yield obtained by harvesting at full flowering stage was higher than harvesting at the other stages, i. e. before flowering, at fruit set, and after one month from planting by ٢٢,٣٪, ٢٩,٥ %, and ٣٢,٧ % respectively (Table ١٨).

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Table (١٧) Influence of stages of harvesting and fertilizers type on the total yield of Indigo plant (Kg/ha)

First season ٢٠٠١/٢٠٠٢

Harvesting time	Fertilizers type				
	Control	Chicken manure	٥٠ kg/fed urea	١٠٠ kg/fed urea	means
After one month	٣٩٩٤,٠	٤١٧٩,٨	٤٦٣٤,٧	٥٣٧٤,٠	٤٥٤٥,٦
Before flowering	٥٠١٣,٥	٦٢٠١,٥	٦٧٧٨,٩	٧٦٠٩,٣	٦٤٠٠,٨
Full flowering	٥٦٣٦,٥	٧٠٢٤,٥	٧١٧٢,١	٩٣٧٧,٢	٧٣٠٢,٦
Fruit setting	٤٥٤٦,٠	٤٨٩٠,٦	٥٠٩٦,٤	٦٠٩٦,٤	٥١٥٧,٣

means	٤٧٩٧,٥	٥٥٧٤,١	٥٩٢٠,٥	٧١١٤,٣	
LSD	٣٣٦,٥				
SE ±	١١٦,٤				

Table (١٨) Influence of stages of harvesting and fertilizers type on the total yield of Indigo plant (Kg/ha)

Second season ٢٠٠٢/٢٠٠٣

Harvesting time	Fertilizers type				
	Control	Chicken manure	٥٠ kg/fed urea	١٠٠ kg/fed urea	means
After one month	٤٢٦٠,٧	٥٥٠٢,٠	٦٠٧٦,٩	٦٥٤٥,٨	٥٥٩٦,٤
Before flowering	٥١١٧,٦	٦٢١٦,٦	٦٨٣٥,٤	٧٦٥٥,٧	٦٤٥٦,٣
Full flowering	٦٧٦٧,٠	٧٥١٧,٦	٨٠٠٧,١	١٠٩٥٢,٨	٨٣١١,١
Fruit					

setting	٥٠٧٢,٦	٥٧٥٢,٩	٥٧٢٦,٣	٦٨٩١,٤	٥٨٦٠,٨
means	٥٣٠٤,٥	٦٢٤٧,٣	٦٦٦١,٤	٨٠١١,٤	
LSD	٥٠٨,١				
SE ±	١٧٦,٠				

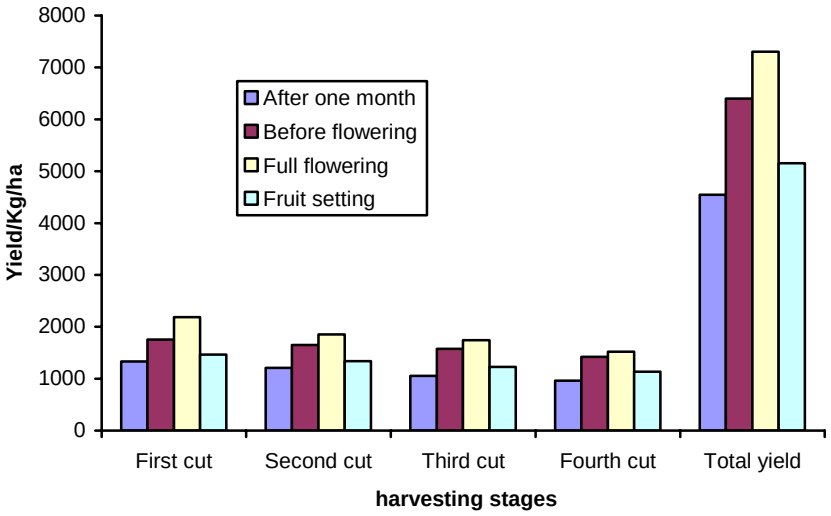


Fig (18) Influence of stages of harvesting time on the yield of Indigo plant (Kg/ha) (First Season 2001/2002)

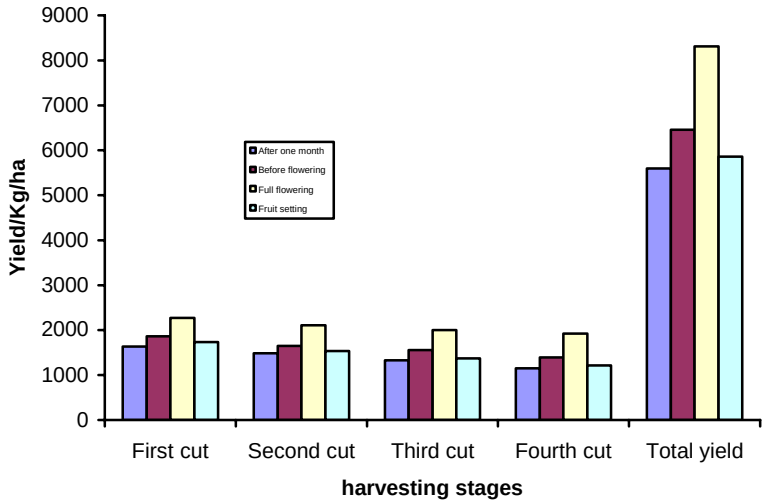


Fig (19) Influence of stages of harvesting time on the yield of Indigo plant (Kg/ha) (second season 2002/2003)

Table (١٩) Influence of stages of harvesting and fertilizers type on the total dye content (%)

First season ٢٠٠١/٢٠٠٢

Harvesting time	Fertilizers type				
	Control	Chicken manure	٥٠ kg/fed urea	١٠٠ kg/fed urea	means

After one month	٤٩,٠	٥٠,٤	٥٣,٥	٥٨,٢	٥٢,٨
Before flowering	٥٢,٠	٥٥,٧	٦١,٦	٦٨,٤	٥٩,٤
Full flowering	٥٣,١	٥٩,٤	٦٦,٩	٧٣,٧	٦٣,٣
Fruit setting	٤٩,٥	٥٣,٥	٥٩,٦	٦٤,٢	٥٦,٧
means	٥٠,٩	٥٤,٧	٦٠,٤	٦٦,١	
LSD	٤,٤				
SE ±	١,٥				

Table (٢٠) Influence of stage of harvesting and fertilizers type on the total dye content (%)

Second season ٢٠٠٢/٢٠٠٣

Harvesting	Fertilizers type	

time	Control	Chicken manure	٥٠ kg/fed urea	١٠٠ kg/fed urea	means
After one month	٤٢,١	٤٥,٣	٥٤,٨	٦٣,٦	٥١,٥
Before flowering	٤٩,٣	٥٢,٥	٦٣,١	٧٠,٧	٥٨,٩
Full flowering	٥٦,٥	٦٣,٢	٧١,٣	٧٥,٩	٦٦,٧
Fruit setting	٥٠,٩	٥٤,٠	٦٥,٤	٧١,٠	٦٠,٣
means	٤٩,٧	٥٣,٧	٦٣,٦	٧٠,٣	
LSD	٧,٩				٤,٠
SE ±	٢,٧				١,٣

Fig (20) Influence of stages of harvesting time on dye content (%) (First Season 2001/2002)

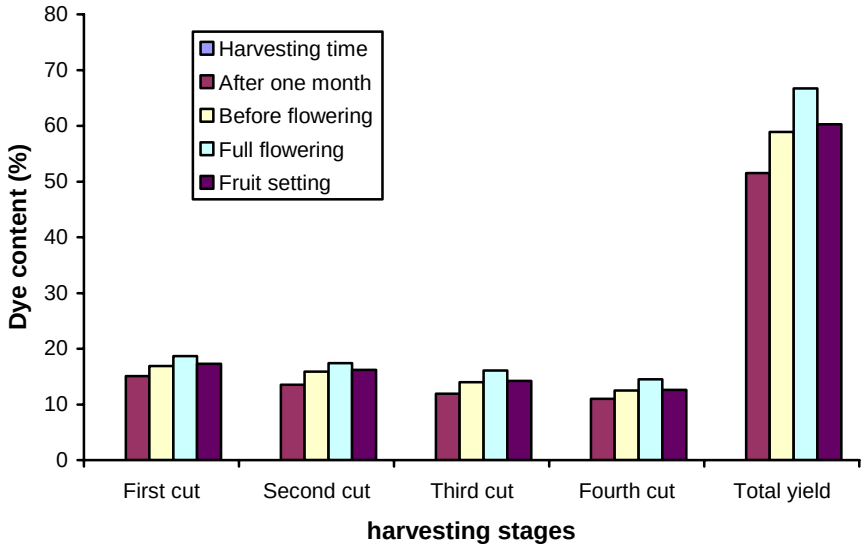


Fig (21) Influence of stage of harvesting time on dye content (%) (Second Season 2002/2003)

Similar results were obtained in the dye content (tables ١٩ and ٢٠). The effect of the stages of harvest on total dye content was highly significant (P=٠,٠١). Plants harvested at full flowering stage gave the highest total dye content and those harvested one month from planting gave the lowest dye content.

In the first season, the first cut produced the highest dye content at full flowering stage. There were significant differences between harvesting before flowering and at fruit setting. Plants harvested one month from planting produced the lowest dye content. In the second cut, there were no significant differences between harvesting before flowering and at full flowering. Dye content obtained from plants harvested at fruit setting was significantly lower than that obtained before flowering and at full flowering. Dye content obtained from plants harvested one month from planting was significantly the lowest compared to other treatments.

In the third cut, the plants harvested at full flowering stage gave the highest dye content, and slight decrease was noted when harvesting was carried out before flowering and at fruit setting, also the lowest dye content was obtained from plants harvested after one month from planting. In the fourth cut highest dye content was obtained from plants harvested before and at full flowering stages, with no significant difference between the two stages. Also, there were no significant differences between the other two stages i.e. after one month from planting and at fruit setting (Fig ٢٠).

In the second season, the same trend was noted in the four cuts. Harvesting at full flowering stage resulted in higher dye content, but there was no significant difference between harvesting before flowering and fruit setting stages in the dye content. The lowest dye content was obtained when the plants were harvested after one month from planting (Fig ٢١).

٦:٥:٢ Effect of fertilizers type on yield and dye content of indigo plant

The effect of fertilizer application was highly significant in the four cuts, (P=٠,٠١), on yield and dye content in both seasons. The control attained a significantly lower yield and dye content than the rest of the treatments.

In the first season as shown in (Fig ٢٢) gave the highest yield when Nitrogen fertilizer was applied at ١٠٠ kg/fed. There were no significant differences between chicken manure and nitrogen fertilizer at ٥٠ kg/fed. The control gave the lowest yield. The highest total yield was obtained by ١٠٠ kg/fed urea, followed by ٥٠ kg/fed, chicken manure, and the lowest yield was obtained from the control.

Fertilizer treatment at ١٠٠ kg/fed urea out- yielded other treatments in total yield in the first season. i.e. ٥٠ kg/fed urea, ٢,٥ ton/fed composted chicken manure and the control by ١٦,٨٪, ٢١,٧ % and ٣٢,٦ % respectively (table ١٧).

The same trend was obtained in the second season where the highest total yield was obtained by addition of ١٠٠ kg/fed urea. In the

four cuts (Fig ٢٣), the highest yield was obtained from plots receiving ١٠٠ kg/fed urea. There were no significant differences between ٥٠ kg/fed urea and composted chicken manure; the control gave the lowest yield in the four cuts. Fertilizer at ١٠٠ kg urea/fed gave higher total yield than other treatments by ١٦,٩ % ٢٪ and ٣٣,٨ % compared

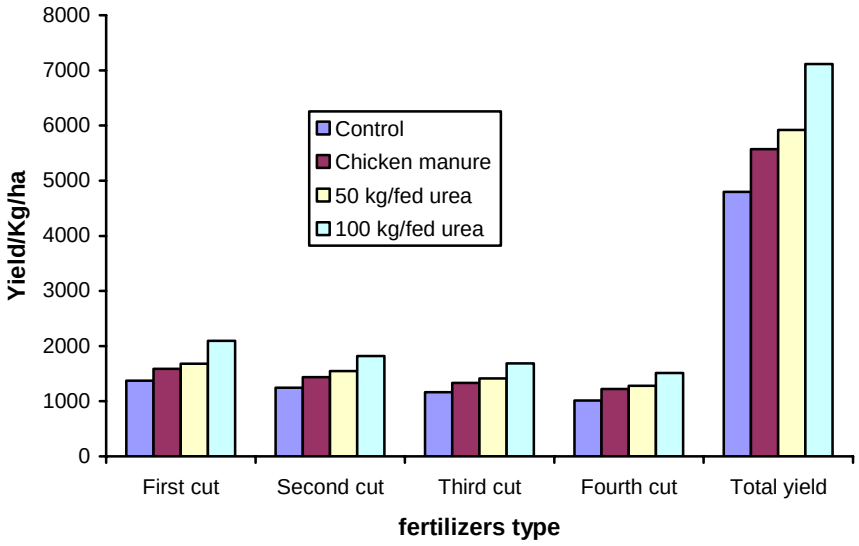


Fig (22) Influence of the fertilizers type on the yield of Indigo plant (Kg/ha) (First Season 2001/2002)

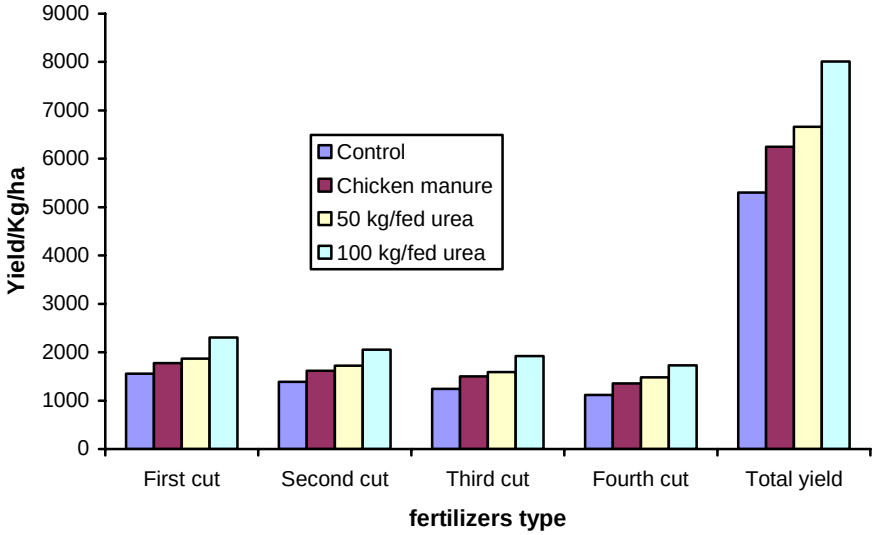


Fig (23) Influence of fertilizers type on the yield of indigo plant (Kg/ha) (Second Season 2002/2003)

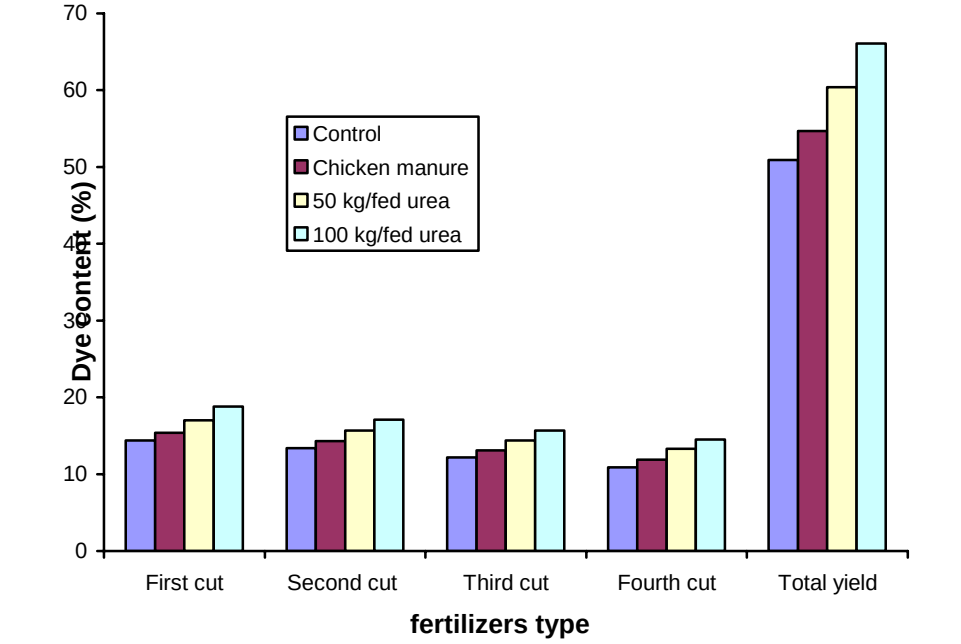


Fig (24) Influence of the fertilizers type on dye content (%) (First Season 2001/2002)

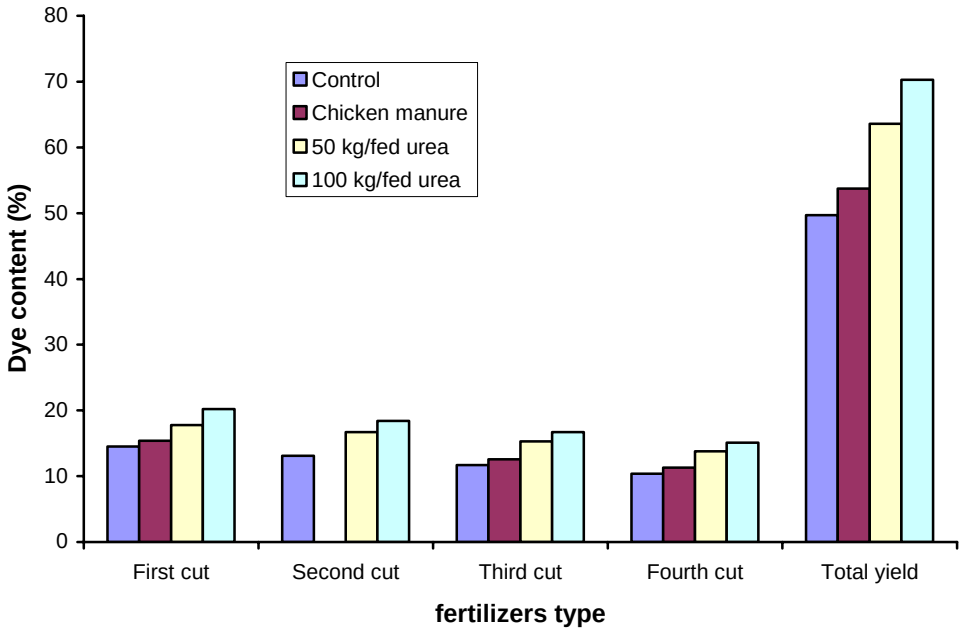


Fig (25) Influence of fertilizers type on dye content (%) (Second Season 2002/2003)

with ٥٠ kg/fed urea, chicken manure and the control respectively (Table ١٨).

The total dye content was highly significantly affected by fertilizer treatments (p=٠,٠١) (table ١٩ and ٢٠). Urea at ١٠٠ kg/fed gave higher dye content than the other treatments. In the first season, the highest dye content in the four cuts was obtained by application of ١٠٠ kg/fed urea, followed by ٥٠ kg/fed urea. There were no significant differences between composted chicken manure and the control (Fig ٢٤).

Similar results were obtained in the second season. Differences in dye content were associated with differences in yield. With the exception of the fourth cut the highest dye content was obtained by ١٠٠ kg/fed urea, followed by ٥٠ kg/fed urea, chicken manure and the control. It was clear that both yield and dye content responded similarly to the application of fertilizers (Fig ٢٥).

Interactions between treatments in the first season were not significant in three cuts, however, in the fourth cut and total yield, there were significant differences (p=٠,٠٥). Harvesting plants at full flowering stage and addition of ١٠٠ kg/fed urea gave the highest total yield (table ١٧).

In the second season significant differences in yield were found in the fourth cut due to the interactions between treatments (Table ١٨).

There were no significant differences due to the interactions between treatments in dye content in both seasons.

This experiment was necessitated by the lack of adequate information on fertilizer application and time of harvest of *Indigofera tinctoria*. Hancock, (١٩٩٧) mentioned that the husbandry techniques need to be developed.

It is evident from the results that harvesting at the flowering stage was the best, it gave the highest yield and dye content. This might be attributed to the fact that plants had enough time to produce their maximum vegetative growth at this stage. These results are in line with those of Harvery, (١٨٩٨) and Duke, (١٩٨٣), who reported that the plants are cut during the flowering season. Also in Bangladesh it was reported that the plants flowered during July- September and were harvested during this time. An FAO document (١٩٩٥) reported that the first harvest was taken three or four months from planting when the plants were flowering and this involved cutting the stem ١٠-٢٠ cm above ground level; under favorable conditions three cuts were obtained per year.

Gilber and Cooke (٢٠٠١) reported that time of harvest could have significant effect on the dye content. Also Minami, *et al.,* (٢٠٠٠) stated that the indican content of *Polygonum tinctorium* increased with the maturity of the leaves. While Mairapetyon, *et al*., (١٩٨٦), reported that leaves from the first harvest had maximum indigo content. Testuo, *et al*., (١٩٨١) reported that in the woad plant, the older leaves contained a lower concentration of the indoxyle derivatives up to ١٤٪ dry weight while in the youngest leaves approximately ٢٤٪ of the dry weight was found. In a progress report summary (http://www.) it was found that the number of harvests varied between one and four; also Anna and Christina (٢٠٠٢) observed that the indigo dye in the *Polygonum tinctorium* varied in the

first cutting from the second cutting and the yield of the two cuts was on average ٣,١ t/ha.

Zavatskaya and Mashanova (١٩٧٨) studied the sugar synthesis during plant development in relation to accumulation of the glycoside form of the dye. They found that sugar synthesis was maximal during full bloom when dyeing ability of the leaves was also highest. Also Cano, *et al*., ١٩٩٠, reported that the optimum glycoside constituents in *Cassia* occurred when the plant was ٦٥ days old and decreased when plant age was ١٥٠-١٦٠ days.

It was found that the highest yield and indigo dye in the Woad plant was obtained when the plants were harvested at the end of July compared to the beginning of September (http://www.).

Nitrogen fertilizer in the form of urea at ١٠٠ kg/fed produced highest yield and dye content. Nitrogen is an important element in plant life. It is an indispensable part of protein, chlorophyll, amino acids, nucleic acids, enzymes, and vitamins. It is responsible for vegetative plant growth, root development and the color of the grass (Ahmed, ١٩٩٤). It allows plant leaves to grow longer and hence to have a large surface available for photosynthesis which is roughly proportional to the amount of nitrogen applied. The nitrogen fertilizers therefore markedly affect vigour of plant growth and its photosynthestic ability (Rabih, ١٩٩٩).

FAO document (١٩٩٥) reported that *Indigofera tinctoria* grew best on permeable soil which was rich in organic matter. The response of indigo plant to nitrogen fertilizer was similar to that of other leguminous plants, reported by Mohamed, (١٩٩٠); Nayel, (١٩٨٤); Mohamed (١٩٨٤) and Mohamed, (١٩٧٦). Rabih (١٩٩٩) reported that nitrogen fertilizer

significantly increased yield components of haricot bean. Elamin, (١٩٩٨) found that nitrogen fertilizer resulted in the highest yield of leaves of *Cassia acutifolia*. Field experiment on senna in India showed that application of ١٠ tons farm yard manure/ha improved total leaf and pod yield (Ilongovan, *et al*., ١٩٨٩). On the other hand, Gobara, (١٩٨٨) reported that application of nitrogenous fertilizers to snap bean is common practice, and doses as high as ١٠٠/kg/ha are recommended to obtain reasonable yield.

٤:٢ Laboratory Work

٤:٢:١ Chemical analysis

The extraction of dye content was investigated by using different solvents. The results presented in table (٢١) showed that highest dye content was obtained by using ethanol (٨٠٪) followed by extraction using petroleum ether (b.p ٦٠٪) and ethanol (٨٠٪) successively, petroleum ether (b.p ٦٠٪) and lowest dye content was obtained by using chloroform.

These results were confirmed by Zhang, *et al.*, ١٩٩٠ who reported that the extraction with alcohol can give more indigotin and indirubin, and also Abdamula (٢٠٠٢) who found that alcohol (٩٦٪) is the most effective solvent, which gave higher percentage of extract than chloroform and toluene. Trease and Evans (١٩٧٨) mentioned that alcohol is a general solvent for extraction many plant constituents.

٤:٢:٢ Phytochemical screening

The results obtained from the screening showed that the plant constituents were as shown in table (٢٢). In the test of Unsaturated Sterols and Triterpenses, gradual appearance of green, blue pink to purple color was observed and was taken as an evidence of the presence of Sterols and Triterpenses. A slight turbidity of the two test tubes was obtained and was taken as an evidence for the presence of alkaloids. In the test of flavonids dark yellow color indicated the presence of flavonids compounds. The formation of precipitate was taken as evidence for the presence of tannin in the indigo plant. The appearance of honeycomb which persisted for least an hour was taken as indication for presence of saponins. The test of cyanogenic glycoside was positive that the color of the sodium picrate paper was changed to red color. The anthraquinone glycoside was found that the pink alkaline layer was present. Test for cumarins was negative that the spot was not adsorbed under UV light. These results were in line with the finding of Trease and Evans (١٩٧٨) who reported that the leguminous plants contained constituents of cyanogenic glycoside, saponins, tannins, anthraquinone and alkaloids. El Ghazali *et al*, ٢٠٠٣ reported that amino acids flavonids, alkaloids and indole alkaloids were isolated from *Indigofera tinctoria.*

٤:٢:٣ Thin layer chromatography

Figures (٢٦, ٢٧, ٢٨, ٢٩, ٣٠ and ٣١) showed the bands separated by the solvent system on silica gel glass plates. It was clear that the same bands were appeared in samples, which predict similar compounds of all samples at least for the major compound. TLC technique had shown that

the dye content possess more than three components according to R_f values and color of bands separated (table ٢٣). The colors ranged between blue- violet and R_f ranged between ٠,٢٤-٠,٨٣.

This result was confirmed by Akhmetov, *et al.*, (١٩٨٢) who reported that no dye can gives a pure shade, i.e. dose not reflect only one band of wave length. Christine, *et al.*, (٢٠٠٤) reported that TLC analysis of methanolic extract from leaves of *Isatis tinctoria* showed more than three bands and R_f of indirubin (red) between ٠,٢٥-٠,٣٠ and Abdalmula (٢٠٠٢) reported that the indigo R_f is ٠,٧١.

Table (٢١) Results of extraction with different solvents

Solvents	Dye content%
Ethanol (٨٠٪)	١٧,٧٪
petroleum ether (b.p ٦٠٪) and ethanol (٨٠٪) successively	١٠,٢٪
Petroleum ether (b.p ٦٠٪)	٧,٨٪
Chloroform	٤,٤٪

Table (٢٢) Chemical constituents of Indigo plant

Chemical constituents	Results
Test for Unsaturated Sterols and Triterpenses	+
Test for Alkaloids	+
Test for Flavonoids	+
Test for Tannins	+
Test for Saponins	+
Test for cyanogenic glycoside	+
Test for Anthraquinone glycoside	+
Test for Cumarins	__

Where:

+ Positive result

-Negative result

Table (٢٣) R_f values and colors of the bands

Samples	Color	R_f
cultivated plant	Blue- violet	٠,٢٤-٠٨٣
Wild plant	Blue- violet	٠,٢٣-.٠٨١
local market	Red	٠,٢٢
sowing date X spacing	Blue- violet	٠,٢٣-٠٨٣

stages of harvest X fertilizer	Blue- violet	٠,٢٣-٠٨٢
Water management	Blue- violet	٠,٢٢-٠٨٢

Muagard (٢٠٠١) identified and quantified indigoid pigment, indigo, interurbin, isoindig and isointerurbin and indigo precursors by using HPLC.

٤:٢:٤ Column chromatography

On the basis of U V absorption, fraction (١) ١:٧:١ and fraction (٢) ١:٧:٢ (Fig ٣٢ and ٣٣) were divided into four different bands and then crashed, dissolved in methanol, filtered and filtrates were evaporated in room temperature and chromatographed by TLC . One spot was appeared and

further attempts of purification were not successful and resulted in pale color. This result was confirmed with Christine, *et al.*, (٢٠٠٤) who used column chromatography to purify the indigo precursors and found that the more purification was not successful.

Fig (٢٦) Sample of cultivated plant

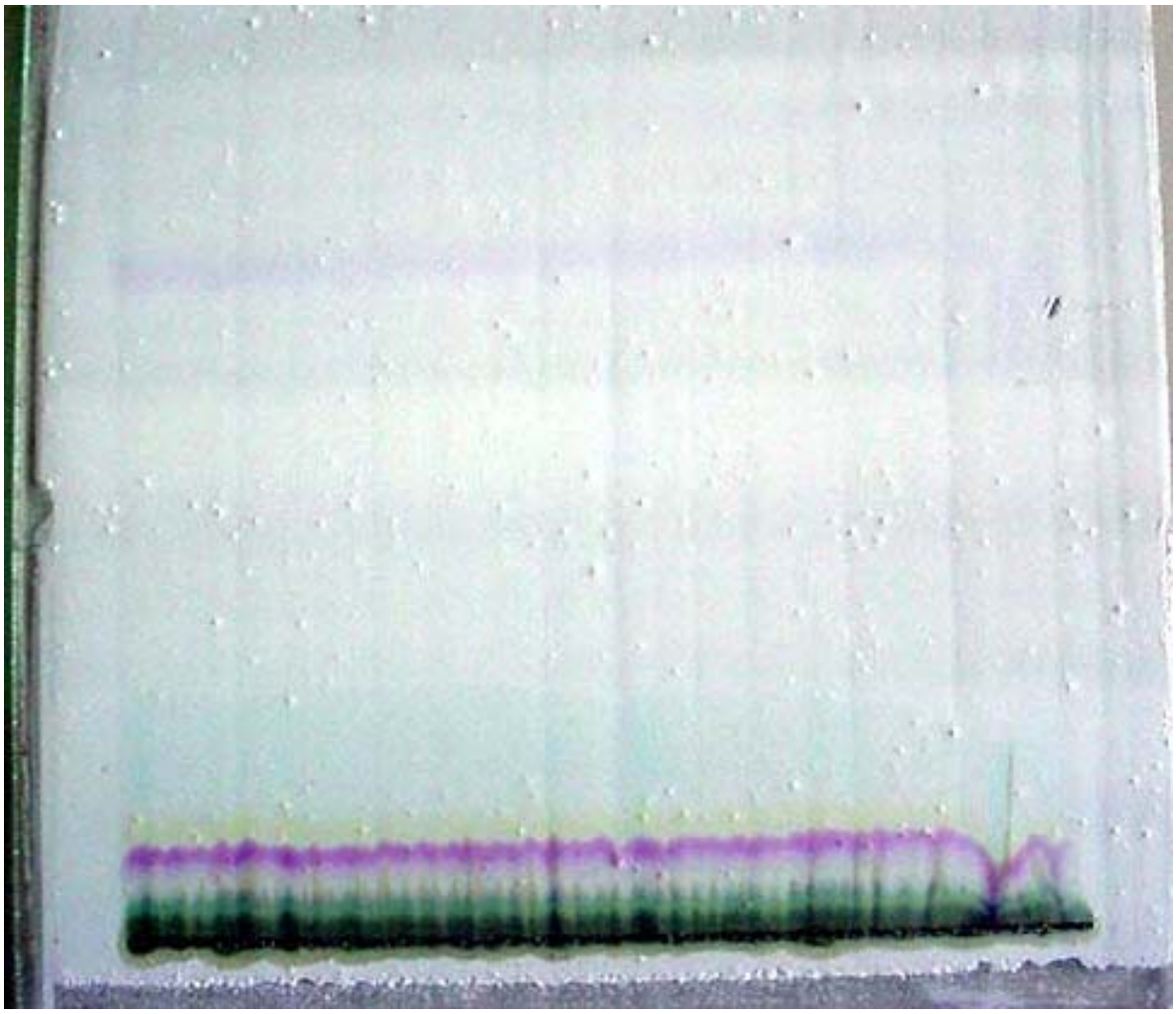


Fig (٢٧) Sample of sowing dates x spacing

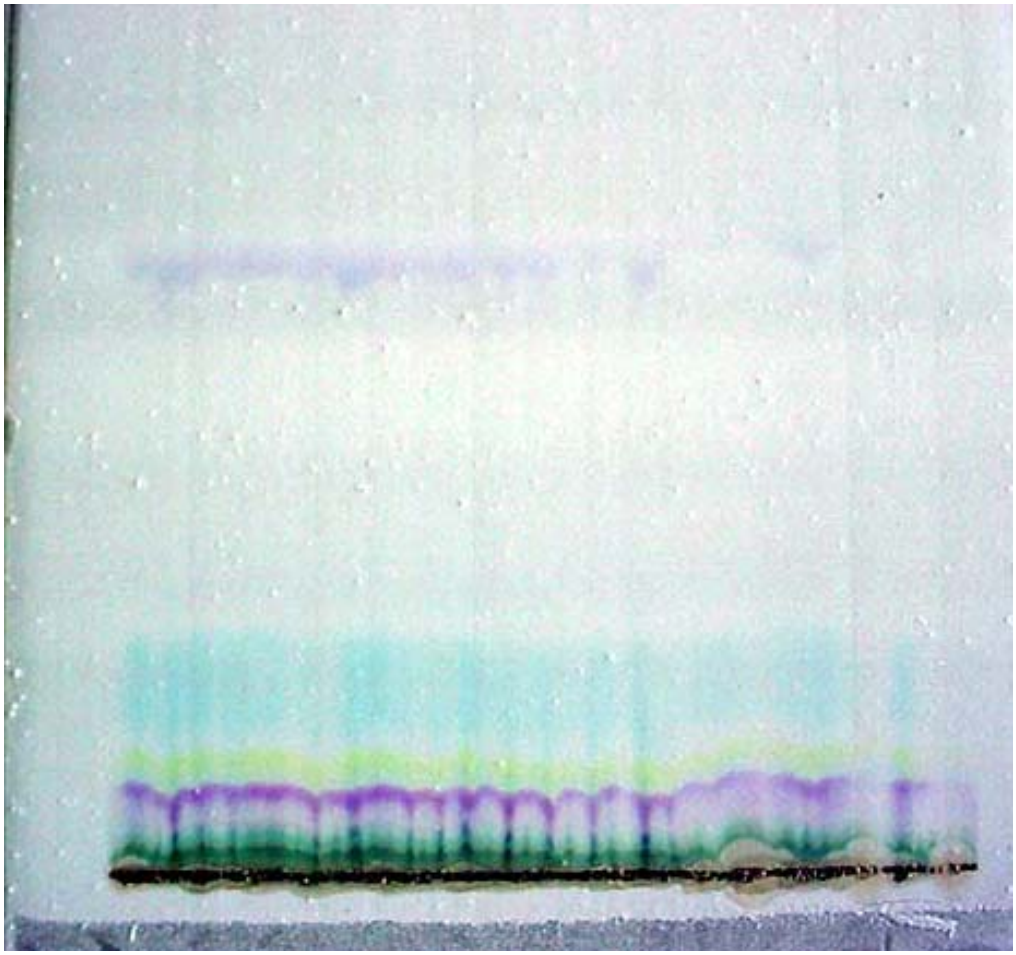


Fig (٢٨) Sample of Wild plant

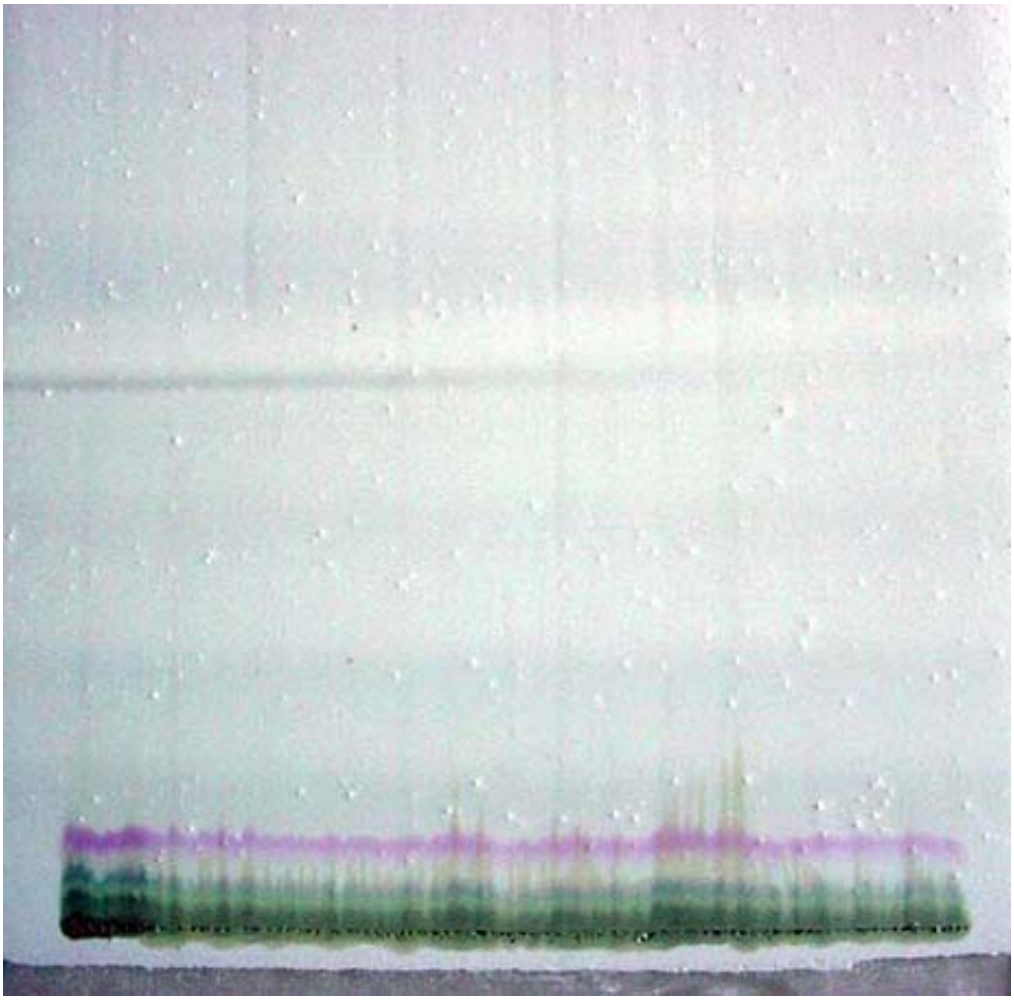


Fig (٢٩) Sample of fertilizers application x stages of harvest

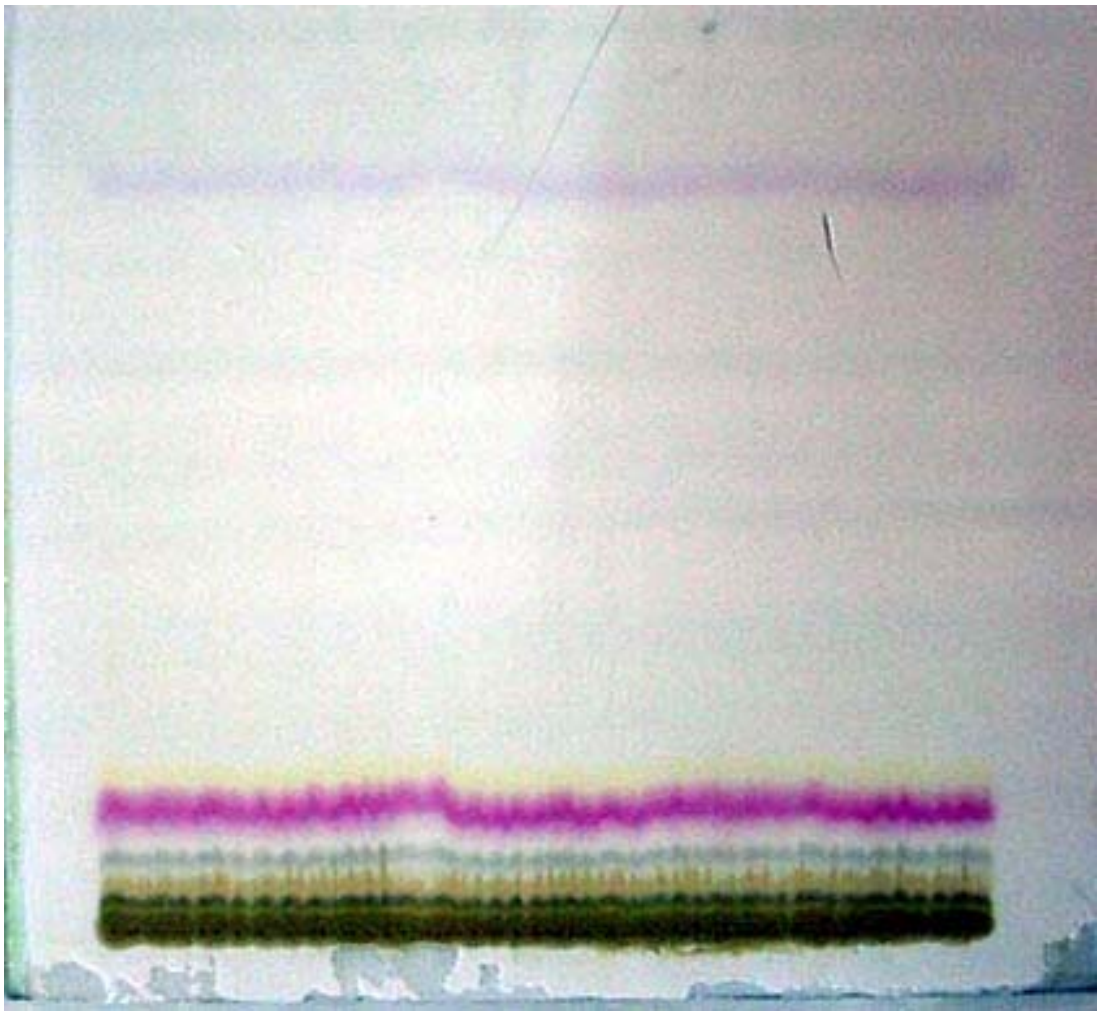


Fig (٣٠) Sample of water management

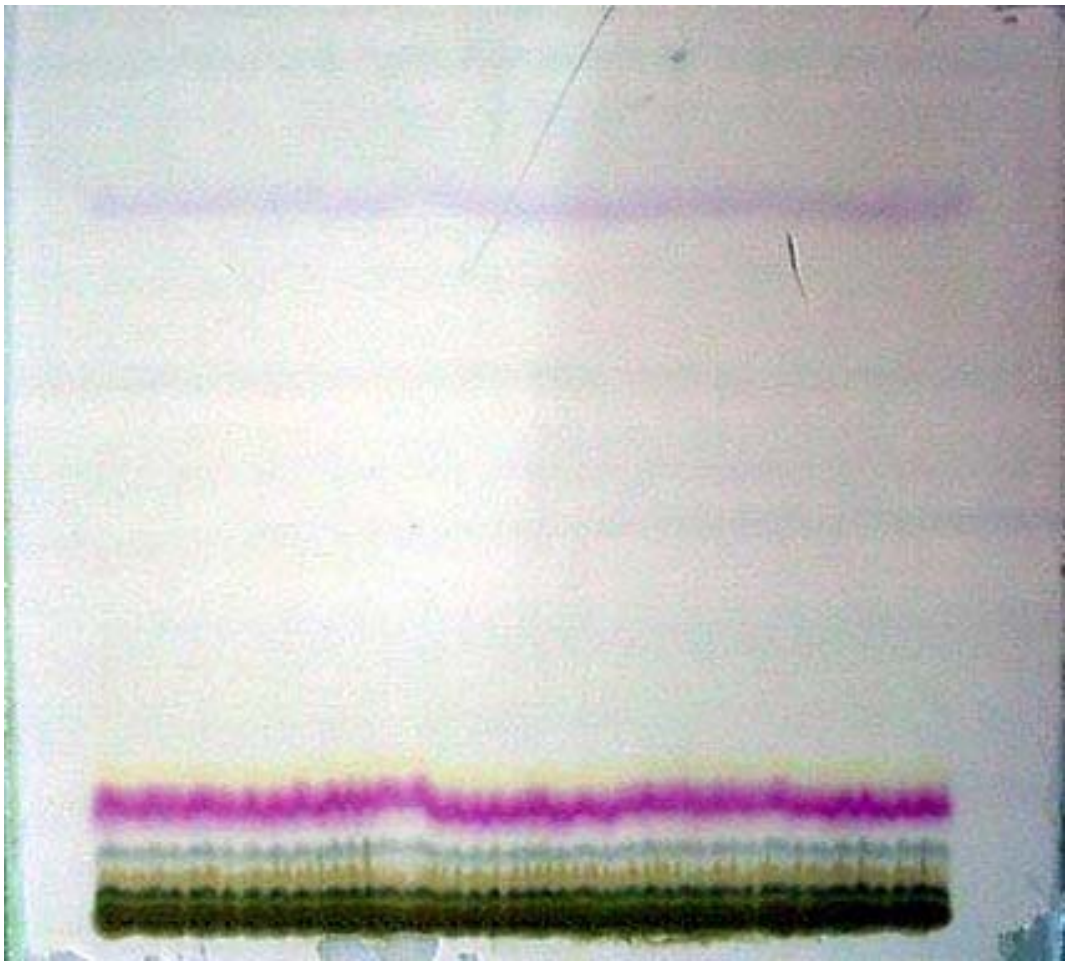


Fig (٣١) Sample of local market

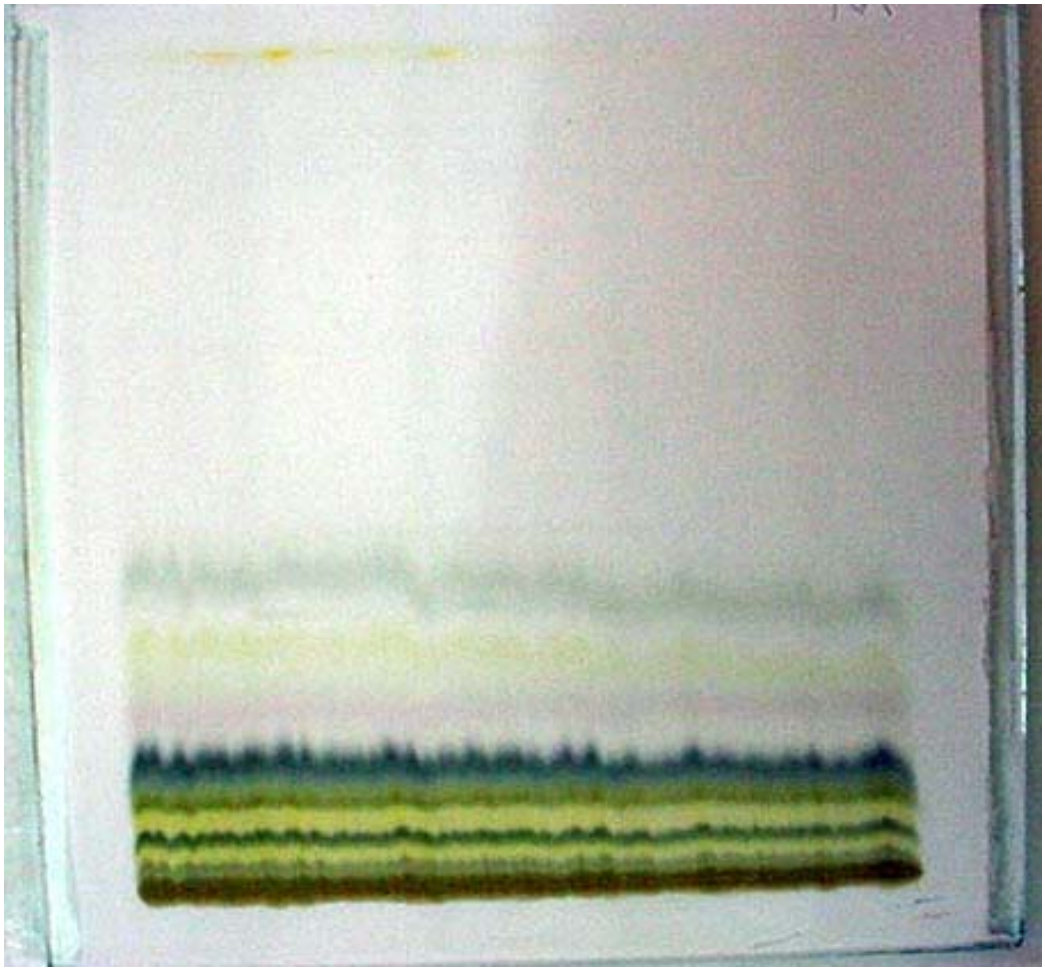


Fig (٣٢) Fraction (١)

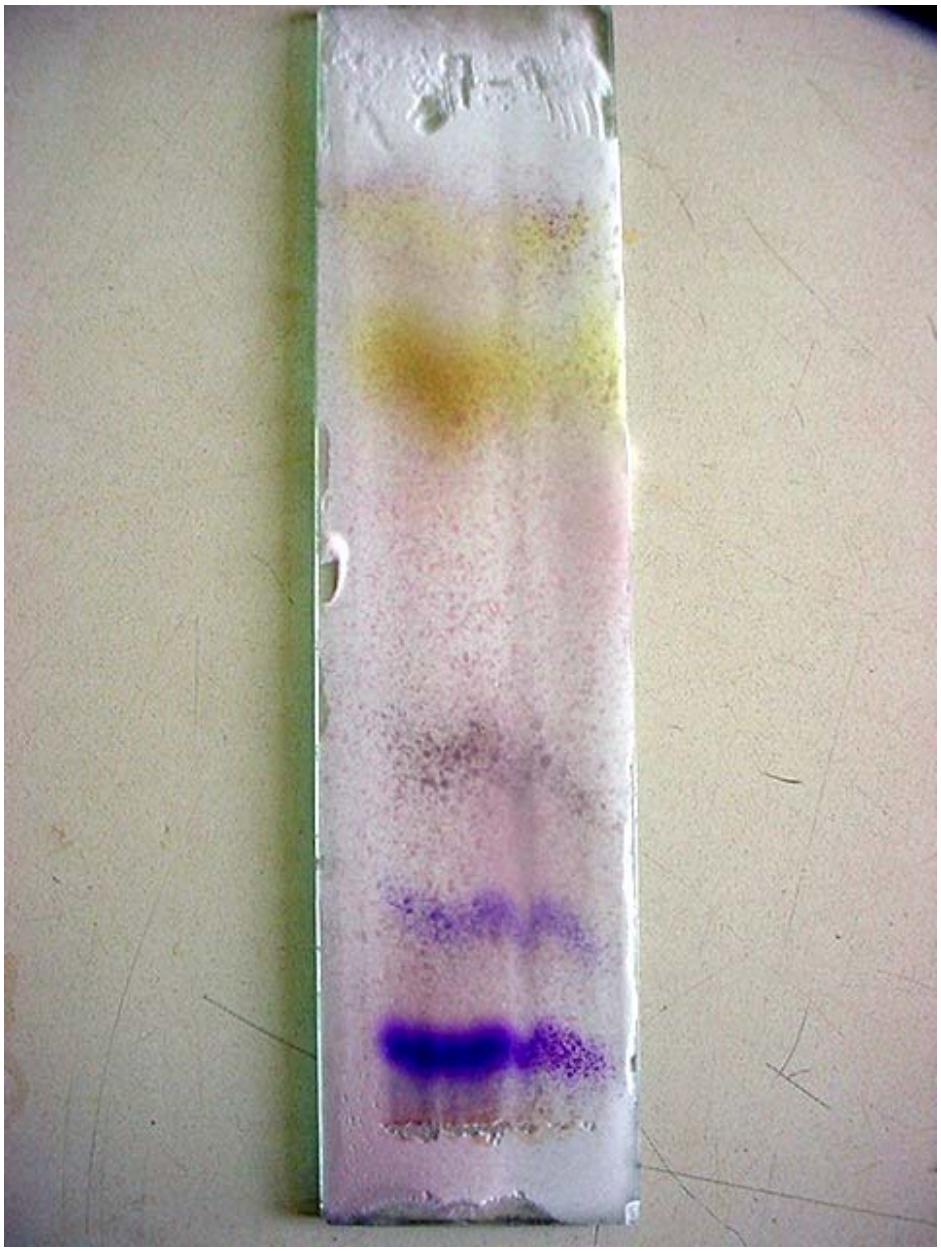
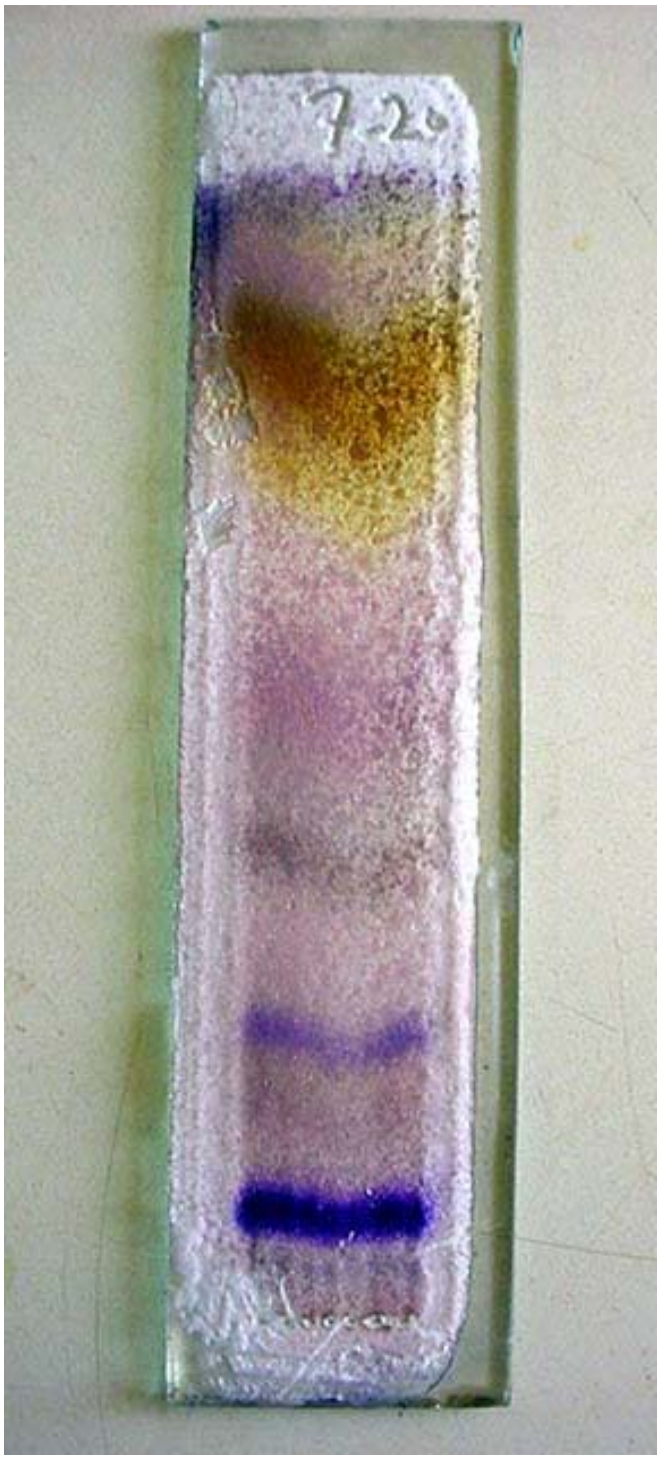


Fig (٣٣) Fraction (٢)



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Appendix (١) Monthly air temperature relative humidity and rainfall during the period of experiments.

Month	Mean temperature ºC		Relative humidity	Total rainfall (mm)	Mean temperature ºC		Relative humidity	Total rainfall (mm
	Max	Min			Max	Min		
	٢٠٠١				٢٠٠٢			
January	٢٩,٧	١٣,٥	٢٦	٠	٢٨,٠	١٢,٧	٢٦	٠
February	٣١,٩	١٤,٨	٢٣	٠	٣٣,٦	٢٦,٦	٢٤	٠
March	٣٧,٣	١٨,٣	١٦	TR	٣٦,٧	١٧,٣	١٧	٠
April	٤١,٢	٢٢,٧	١٤	٠	٤٠,٨	٢٠,٧	١٤	٠
May	٤٢,٠	٢٥,١	١٧	TR	٤١,١	٢٤,٧	١٩	٠
June	٤١,١	٢٤,٧	٢١	٠	٤١,٩	٢٦,٢	٢٣	٠,١
July	٣٧,٨	٢٥,٥	٤٠	٤٧,٢	٤١,٥	٢٦,٨	٣٦	٩,٠
August	٣٥,٥	٢٥,٣	٥٥	٧٠,٠	٣٧,٣	٢٥,٢	٤٨	٨٨,٠
September	٣٨,٩	٢٦,٤	٤٣	٦,١	٣٩,٦	٢٦,١	٤٢	١٥,٠
October	٣٩,٤	٢٤,٤	٢٩	٥,١	٣٩,٧	٢٤,٨	٣٠	٦,١
November	٣٥,٧	٢١,١	٢٩	٠	٣٦,١	٢٠,٣	٢٩	٠
December	٣٢,٦	١٦,٦	٢٩	٠	٣٠,٣	١٣,٧	٢٧	٠
	٣٠٠٣							
January	٣١,٧	١٤,٢	٢٧	٠				
February	٣٣,٥	١٥,٥	١٦	٠				
March	٣٥,٨	١٨,٦	١٥	٠				
April	٤٠,٦	٢١,٣	١٦	٠				
May	٤١,٩	٢٥,٨	٢١	٢٦,٢				
June	٤٠,٩	٢٧,٠	٣٣	٦,٠				
July	٣٧,٣	٢٥,٢	٤٩	٤١,١				
August	٣٥,٨	٢٥,٣	٥٨	٧٤,٠				
September	٣٨,٩	٢٥,٢	٤٥	١٢,٩				
October	٣٩,٩	٢٣,٩	٣١	٣,٤				
November	٣٦,١	٢٠,٣	٢٩	٠				
December	٣١,٥	١٥,١	٣٣	٠				

Appendix (٢)

ABBREVIATION

m	meter
cm	centimeter
dm	decimeter
kg	kilogram
ha	hectare
g	gram
R_f	mobility relative to front
U V	Ultra violet
TLC	Thin layer chromatography
HPLC	High performance liquid chromatography
nm	nanometres
b.p	Boiling point