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Physiological Maturity and Harvesting Date on Seed Yield and Quality of Indigo (*Indigofera suffruticosa* Mill.)

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ABSTRACT

Background: Indigo is used to dye many fabrics, which also needs a lot of indigo plants enough to dye the fabric. Indigo seeds were used for propagation, but the seed of indigo is not simultaneous. The physiological maturity and optimum harvesting date affected the yield and quality of indigo seeds. This research aims to study the physiological maturity and harvesting date of indigo on seed yield and quality.

Methods: Indigo plants were grown in the dry season. The experiment was a randomized complete block design (RCBD) with 4 replications, consisting of 4 harvesting dates; 140, 150, 160 and 170 days after planting (DAP).

Result: The results showed that the first indigo flower was at 51 DAP and matured to pods at 59 DAP. It takes 113 DAP for the indigo color to change from green to brown. The appearance of the pods changed from green to yellowish green, brown and black, respectively. As the indigo pods became 50% brown, they started harvesting. The weights of a dry pod, a fresh pod and a seed were different. When harvesting at 140 DAP, the fresh pod weight had the maximum of 82.60 g/plant and dropped as the indigo harvesting date grew ($P<0.01$). The dry pod weight was the lowest, at 27.58 g/plant ($P<0.05$). The seed weight per plant for harvesting at 170 DAP was 25.57 g. The harvesting date, however, did not differ statistically. The highest percentage of germination and vigor were from the indigo seeds, which were harvested at 140 DAP, 31.94% and 38.81%, respectively ($P<0.01$). Therefore, the optimum harvesting date at 140 DAP is the physiological maturity stage and harvesting of the pods when they turn brown for the highest seed quality.

Key words: Germination, Harvesting date, Indigo, Physiological maturity, Seed.

INTRODUCTION

Indigo is a family of Leguminosae found in 30 species in Thailand (Mattapha and Chanttaranothal, 2012). *Indigofera tinctoria* and *Indigofera suffruticosa* are durable and have a distinctive color due to indigo's unique properties as the Queen of Colors (Saithong, 2010). In Sakon Nakhon province in the northeast of Thailand, people have been planting and indigo dyeing for a long time (Panngon *et al.,* 2011; Teanglum, 2012). It has been developing into a natural indigo-dyed cotton fabric that has received a Geographical Indication (GI). It is also a favorite product inside and outside the country (Saithong, 2010; Wiwachan *et al.,* 2015). Indigo seeds need to be planted to have enough indigo for dyeing indigo fabric. However, indigo is a plant that has seed maturation that is not simultaneous. As a result, the pods are not mature at the same time. The pods at the bottom of the tree are getting mature first, while the pods above are still green. Harvesting too early may result in immature seeds and seeds that are harvested later have a higher chance of becoming hard. This impacts pod weight, seed weight, germination percentage and seed vigor and leads to lower production and low-quality seeds (Somrug *et al.,* 2020). When producing seeds, harvesting at the right time is crucial to getting high-vigor seeds (Aswin *et al.,* 2023). The harvesting date has a direct impact on the quantity and quality of seed output (Chanprasert, 2010). The best time to harvest must occur during this phase because seed maturity affects seed quality. Harvesting at physiological maturity, the ideal period, is one method of obtaining high-quality seeds. Based on studies done on those soybean seeds, avoid harvesting black soybean seeds (Lee *et al.,* 2013). The first stage is when pods become brown at 50% and the second is when the pods turn brown at 95% (Thongsri *et al.,* 2015). Regarding peanuts, the pods are dry inside and change color until the pattern can be seen clearly (Teekachunhatean, 1997) and mature seeds had higher emergence, canopy coverage and seed quality than those of immature seeds (Song *et al.,* 2022). Catingueira [*Poincianella pyramidalis* (Tul.) L.P. Queriroz], which are

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legumes, the pods turn light brown, which indicates the physiological maturation stage (Lima *et al.,* 2012). Also, the harvest of sunn hemp (*Crotalaria juncea*) occurs when the pods begin to dry and turn brown and the seeds turn brown (Promsuk *et al.,* 2010; Yonpaladyot *et al.,* 2015) and is carried out at the point where the plants have between 80 and 100% dry pods (Araújo *et al.,* 2018). For peas, they indicated that spot picking of physiologically mature seeds should be done instead of a single harvest (Ayyub *et al.,* 2007). For the harvesting date of jack bean (*Canavalia ensiformis*), the suitable harvesting time was 160 days after planting (Jantabout and Tiamtaisong, 2011). The seed groundnut harvested 135 days after sowing produced seeds with good quality in germination and vigor index (Sharma *et al.,* 2013). The kenaf (*Hibiscus canabinus*) was harvested 35 days after flowering (Olasoji *et al.,* 2012). Fully ripe fruits of rambutan had better seed vigor and seedling growth than half-ripe fruits and green stage (Tetteh *et al.,* 2023) and the germination and seedling growth of yellow seeds were much better than dark brown, more beneficial for seed storage of alfalfa (Gao *et al.,* 2023). Also, seed coat color of cowpea had significant effect on seed vigor and germination percentage after storage (Tetteh *et al.,* 2023). The gambir seeds harvested between 98 and 100 DAA, indicating that the plant had reached its physiological maturity (Zainal *et al.,* 2023). Therefore, harvesting too early or too late will affect the yield and quality of the seeds.

Harvesting at the optimal time is essential to avoid the collection of immature seeds or the loss of seed yields. This study aims to identify the physiological maturity of indigo, the optimal harvesting date for yieldand the quality of indigo seeds.

MATERIALS AND METHODS

Experimental site, experimental design and treatments

The experiment was conducted at the Plant Science. Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon Province, Thailand. The experiment was a randomized complete block design (RCBD) with 4 replications and 4 treatments as harvesting dates: 140, 150, 160 and 170 days after planting (DAP).

Indigo planting

The dry season was used to cultivate indigo plants from June 2015 to February 2016. Indigo seeds were put in a seedling tray using a growing media (peat moss). And 30 days after sowing, the indigo was divided into 16 plots, each measuring 2.5 by 2.5 m. The indigo seedlings were in 4 planting rows and the spacing between plants and rows was 30×60 cm.

Data collection

Data were collected in three stages: 1. flowering stage: data were collected on the date of the first flowering, the first flower bloom, 50% flower blooming and the plant height. 2. pod stage: data were collected on the date of the first pod appear and maturing first pod; the date of the first pod changed color and 3. harvesting stage: data were collected by measuring the height of plants, pod dry weight (brown color), pod fresh weight (green color), number of seeds per pod, 1,000 seed weight, seed weight per plant, percentage of moisture, germination and vigor of the seeds as follows:

Seed moisture

Indigo seeds were weighed for 5 grams with 2 replications. Put the seeds in the hot air oven at 103°C for 17 hours and calculate the moisture percentage using the following formula (ISTA, 2003).

$$\text{Seed moisture (\%)} = \frac{\text{Fresh weight-Dry weight}}{\text{Fresh weight}} \times 100$$

Seed germination

Indigo seeds were tested using the paper method (BP), with 100 seeds per replication and 4 replications. Kept them at room temperature; germination was counted after 4 and 7 days after sowing. The number of normal seedlings, abnormal seedlings, hard seeds, fresh un-germinated seeds and dead seeds was countedand then the seed germination percentage was calculated according to the following formula (ISTA, 2003).

$$\text{Seed germination (\%)} = \frac{\text{Total number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

Seed vigor

Indigo seeds were tested by the accelerated aging test (AA test) method for 4 replications. 100 indigo seeds were put in a plastic box containing water; the seeds were on wire gauze. Then cover the plastic box lid and put the box into the hot air oven at 41°C for 72 hours. The seeds were tested for germination according to the standard method to determine seed vigor (ISTA, 2003).

Data analysis

The data were statistically analyzed using the ANOVA technique. The mean was separated using Duncan's New Multiple Range Test (DMRT) in SAS program version 9.1.

RESULTS AND DISCUSSION

Physiological maturity and yield

Physiological maturity

Indigo seedlings (30 days) were transplanted (Fig 1a) into the field. Data were collected for flowering until seed development. It was found that the first flower of indigo started at 51 DAP and flowers bloomed at 54 DAP. The flowers are pink and bloom from the bottom to the top of the plant (Fig 1b) and the flowers bloom 50% at 76 DAP. It has an average height of 35.69 cm. For seed development, it was found that the small green pods were at 59 DAP and

changed to the brown pod at 113 DAP. The physiological maturity of the indigo pod was changed from green to yellowish green, brown and black, respectively (Fig 1c). Also, the indigo seeds will change according to the pod color, from green to yellowish green to brown and black, respectively. There are 4-5 seeds per pod, small squares with green-brown and dark brown colors (Fig 1d).

Plant height

The indigo pod color changed from green to brown in about 50% of the plant. Start harvesting at 140, 150, 160 and 170 DAP, respectively. The height of indigo was 96.70, 94.20, 93.08 and 93.73 cm, respectively, with no statistical difference (Table 1).

Pod fresh weight and pod dry weight

As the harvesting date became late, the fresh weight of the pods dropped ($P < 0.01$); they were 82.60, 15.29, 2.68 and 0.00 g/plant. In that order, more harvesting dates were associated with increased pod dry weights. Table 1 shows that they were 27.58, 50.67, 54.07 and 51.31 g/plant ($P<0.05$). The maximum total pod weight, 110.18 g/plant, was harvested at 140 DAP (Table 1).

Number of seeds per pod

Indigo had 4.10-4.45 seeds per pod. From 140 and 150 DAP, the highest number of seeds per pod (4.45) was obtained. From 170 DAP, the minimal number of seeds per pod (4.10) was found. Non-significant results were found in a number of seed per pod (Table 1).

Seed weight per plant and 1,000 seeds weight

Indigo at 170 DAP had the highest seed weight of 25.57 g/ plant, followed by indigo at 160, 150 and 140 DAP. There were 23.76, 20.81 and 10.56 g/plant, respectively (Table 2). The differences between harvesting dates for 1,000 seeds were significant ($P < 0.05$). It was found that indigo seeds harvested at 140 DAP had the highest weight of 4.47 g, while indigo seeds harvested at 170 DAP had the lowest weight of 3.74 g (Table 1).

As a result, the pods are not mature at the same time. The pods at the bottom of the tree are getting mature first, while the pods above are still green. In the dry season, indigo will bloom faster than in the rainy season. This results in less productivity and low-quality seeds and affects plant height, pod weight, seed weight, germination percentageand seed vigor (Somrug *et al.,* 2020) because indigo is a plant that needs much water to grow (Saithong, 2010). Also, indigos cultivated in June (rainy season) had a fresh weight of 2,560 kg/rai more than in April (dry season), which had 1,920 kg/rai (Srisamran *et al.,* 2015). Indigo and mulberry were planted with an intercropping system in areas with high rainfall and will yield 1,882 kg/rai (Wiwacharn *et al.,* 2015). It showed that indigo is a plant that needs a lot of water from the beginning of planting until harvesting. Similarly, soybeans had a higher yield obtained when suitable temperature conditions were the seeding date (Karaaslan *et al.,* 2012).

Physiologically, at maturity, the color of the indigo pods changes from green to yellowish green, brownand black and turns more black. The maximum fresh pod weight at 140 DAP was 82.60 g/plant. After that, it will gradually decrease. They resulted in increased dry pod weight. Dry pod weight was maximum at 170 DAP. Also, the sunn hemp pod fresh weight decreased and the dry pod weight increased according to the harvesting date (Yonpaladyot *et al.,* 2015). Similarly, the seed weight and moisture of indigo seeds will be maximum at 140 DAP and gradually decrease afterward. According to *Caesalpinia pulcherrima* (L.) Sw., a legume plant, it was found that the maximum number of seeds in the plants after 35 days after flowering is also when the pod color changes from green to light brownand seed weight and moisture will gradually decrease. The high respiration of the seed had an impact on them (Prajith *et al.,* 2014). In addition, when the seeds' weight decreases, the seeds' moisture content decreases. It is the development pattern of most seeds in legumes (Promsuk *et al.,* 2010; Yonpaladyot *et al.,* 2015; Araújo *et al.,* 2018). The plant has a different symbol indicating the maturity stage of the seed; corn and sorghum have a black layer at the seed. Peanuts have a seed coat wrinkle of the pod dries and changes color. Cucumber fruits turn dark

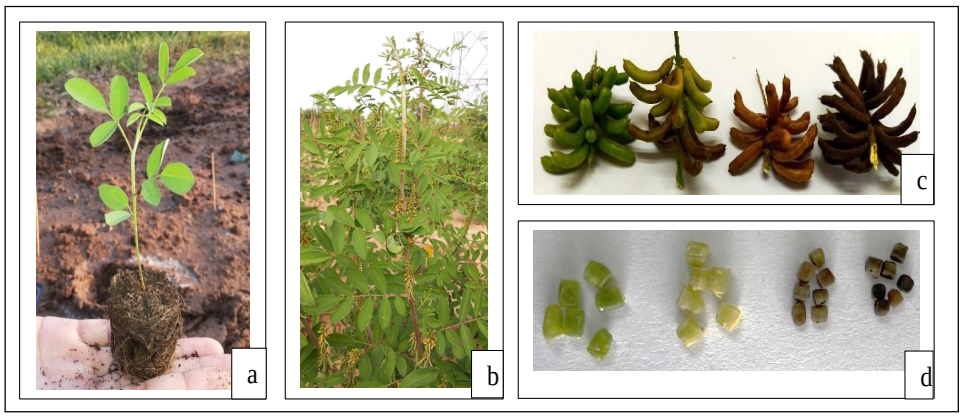

Fig 1: Indigo seedling at 30 days (a), flowering (b), pods (c) and (d) seeds of indigo.

Table 1: Effect of harvesting date on plant height, pod fresh weight, pod dry weight, total pod weight, number of seeds, 1,000 seed weight and seed weight/plant.

Harvesting date	Plant height (cm)	Pod fresh weight/plant (g)	Pod dry weight/plant (g)	Total pod weight/plant (g)	No. of seed/pod	1,000 seed weight (g)	Seed weight /plant(g)
140	96.70	82.60a	27.58b	110.18a	4.45	4.47a	10.56
150	94.20	15.29b	50.67a	65.97b	4.45	3.90b	20.81
160	93.08	2.68b	54.07a	56.76b	4.25	3.88b	23.76
170	93.73	0.00b	51.31a	51.31b	4.10	3.74b	25.57
F-test	ns	**	*	**	ns	*	ns
CV (%)	7.68	72.73	30.33	25.96	5.92	6.25	38.12

Mean with a column followed by the same letter are not significantly different by DMRT, ns: Non-significant at p>0.05, *Significant at p<0.05 and **Significant at p<0.01.

Table 2: Effect of harvesting date on seed moisture, germination, seed vigor, abnormal seedling, fresh un-germinated seed, hard seed and dead seed.

Harvesting date	Seed moisture (%)	Germination (%)	Abnormal seedling (%)	Fresh un-germinated seed(%)	Hard seed (%)	Dead seed (%)	Seed vigor(%)
140	7.02	31.94a	5.25	2.50	42.31c	18.06	38.81a
150	7.19	22.94b	7.50	2.31	53.94bc	13.31	38.50a
160	5.15	18.31b	3.88	1.69	73.94a	3.56	27.50b
170	6.47	19.50b	3.94	1.50	66.94ab	4.94	23.94b
F-test	ns	**	ns	ns	*	ns	**
CV (%)	18.72	16.25	42.09	49.74	15.57	94.57	15.80

Mean with a column followed by the same letter are not significantly different by DMRT, ns: Non-significant at p>0.05, *Significant at p<0.05 and **Significant at p<0.01.

yellowand the calyx is dry (Teekachunhatean, 1997). Sunn hemp seeds have the highest germination percentage (96.50%) at the physiological maturity stage 37 days after flowering; the pods begin to dry, turn brownand the seeds are brown. (Promsuk *et al.,* 2010; Olasoji *et al.,* 2012; Song *et al.,* 2022).

Quality of indigo seeds

Seed moisture

The indigo seeds had a moisture percentage between 5.15% -7.19%. Harvesting at 150 DAP had the highest moisture percentage, followed by indigo seeds harvested at 140, 170 and 160 DAP. The moisture percentages were 7.02%, 6.47% and 5.15%, respectively, with no statistical difference (Table 2).

Seed germination

Indigo seeds harvested at 140 DAP had the highest germination percentage of 31.94%, followed by harvested at 150, 170 and 160 DAP, where there was 22.94%, 19.50% and 18.31%, respectively (P<0.01). The percentage of germination decreases with increased hard seeds (Table 2).

Seed vigor

The vigor percentage was 23.94-38.81% after the accelerating ageing method (P <0.01). Indigo seeds harvested at 150 DAP (38.50%) had the highest seed vigor, while seeds harvested at 170 DAP (23.94%) had the lowest seed vigor (Table 2).

For the harvesting date, the seeds of indigo have a higher percentage of germination and seed vigor when harvested at 140 DAP, but the yield will be the lowest. Indigo seeds' germination percentage and seed vigor decreased when indigo was harvested at 160 and 170 DAP; the indigo pods changed from brown to black and indigo seeds were hard. Therefore, they have the lowest germination (18.35%). Similarly, Yonpaladyot *et al.* (2015) and Promsuk *et al.* (2010) reported that seeds of sunn hemp at 35 days after flowering, they were maximum hard seeds, resulting in the lowest germination percentage and the moisture of the seeds decreased. The results relate to the dormancy of seeds, which shows that seed development is represented in the form of dry matter accumulation (Araújo *et al.,* 2018). Indigo at 170 DAP had a lot of dry pods, but seeds were a low percentage of germination and vigor, which are 19.50% and 23.94%, respectively. However, indigo at 140 DAP was the highest percentage of germination and vigor, which were 31.94% and 38.81%, respectively. According to the study of Jaihan (2008), indigo seeds harvested from the 50% maturity stage had a higher percentage of germination than seeds harvested from the 100% maturity stage, with a germination percentage of 47.25 and 30.00%, respectively. According to the vigor, the seeds were 65.25% and 49.50%, respectively. Therefore, the seeds should be harvested at the physiological maturity stage (PM), which is the stage in which seeds have the highest percentage of germination

and vigor (Chanprsert, 2010). If this stage is passed, it can lead to the degradation of the seed quality (Ayyub *et al.,* 2007; Sharma *et al.,* 2013; Lee *et al.,* 2013).

CONCLUSION

From this study it could be concluded that the first indigo flowered at 51 DAP and flowers bloomed at 54 DAP and changed to the pod at 59 DAP, during this stage the shape of the pods is globular, bent and short. The pod color changes from green to brown at 113 DAP. Then the pods turn increasingly black according to the harvesting date of the indigo. Also, the color of indigo seeds will turn from green to yellow, brown and black respectively. The shape of indigo seeds is a small square. Therefore, the harvesting date of indigo was 140 DAP and dry brown pods were harvested for the highest quality of seed.

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Conflict of interest

This manuscript has no conflict of interest. It no financial support or benefits have been received by myself, by any author.

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