The Pharma Innovation Journal 2022; 11(7): 3599-3605

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(7): 3599-3605

www.thepharmajournal.com
Received: 17-03-2022
Accepted: 23-06-2022

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Tracing the pattern of changes in Physio-chemical properties of seed during development in nightshade (*Solanum trilobatum* L.)

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Abstract
Nightshade (*Solanum Trilobatum* L.) is one of the most important and underutilized medicinal plants. The plant grows majorly in all parts of South India. It has a significant number of medicinal properties which helps to cure terrible ailments. Based on its demand in pharmaceutical sector the plant needs to be cultivated, so the factors governing seed quality needs to be analysed. The study was designed to trace the pattern of seed development and maturation of crop for harvest. The sufficient number of fully opened flowers were tagged at each five days interval to identify the seed development pattern. At each five days interval the berry and seed characters such as fresh weight (g), dry weight (g), moisture content (%), seed recovery (%), 100 seed weight (g) were recorded. The results revealed that the berry and seed attained physiological maturity at 35 days after anthesis (DAA) resulted maximum accumulation of dry matter of both berry (0.84 g) and seed (0.14g) combined with minimum moisture content (49.16% for berry and 15.79% for seed). The berry colour changes from light green to mild red is the physiological maturity indices of nightshade. The fresh seeds were not germinated in any of the development stages.

Keywords: Nightshade, seed development, dry matter accumulation, physiological maturity index

Introduction
India is a primary hub of medicinal plants where large number of medicinal herbs were identified. Medicinal plants contain a large number of primary active ingredients, helps in the preparation of traditional herbal medicines (Dwivedi, 2019) [8]. Several Ayurvedic medicines have been developed using plant extracts having therapeutic value. Antioxidants, anti-inflammatory, anti-insecticidal, antiparasitic, antibiotics, anti-haemolytic characteristics and other therapeutic active compounds were identified in medicinal plants. Even, the world is getting modernized, the usage of herbal medicines is still increasing among the people. There are certain number of unidentified and underutilised medicinal plants are available in our country, these plants need to be conserved and regenerated.

Solanum trilobatum L. is one of the medicinal plants belongs to the family Solanaceae. It is also called as *Alarka* in Sanskrit, *Tuduvalai* in Tamil, *Alarkapatramu* in Telugu, *Tutuvalam* in Malayalam and Purple fruited pea eggplant in English. It is of Indo-Malaysian origin distributed throughout India, primarily in Maharashtra, Kerala, Karnataka and almost all of districts of Tamil Nadu. The leaves, fruits, seeds contain different phytochemicals such as solasoline, sobatum, β-solamarine, solanine, solasodine, glycoalkaloid, disogenin and tomatidine (Chinthana *et al*., 2012) [5]. *S. trilobatum* has antioxidant and hepatoprotective properties (Shahjahan *et al*., 2005) [24]. In siddha, it is used with ghee to cure tuberculosis, as a decoction to treat acute and chronic bronchitis, and the root and berries to treat cough (Swathi *et al*., 2010) [26]. Biological evaluation of the alkaloid mixture of this plant has anticancer activity against specific type of cancer and its effectiveness as an adjuvant in cancer chemotherapy (Kumar *et al*., 2011) [19].

The plant is prickly diffuse, green herb which grows up to 2-3 m height. It possesses spiny branched stem, deltoid or triangular leaves. The flowers are purple coloured cyme and the berries are red.

The morphological and physiological changes that occur from the time of fertilization until the mature ovule (seed) is ready for harvest are referred as seed maturation (Delouche, 1976). The period between fertilisation and maximum dry weight accumulation is referred to as seed development (Abdul Baki and Baker, 1973) [2]. The nature of seed filling is based on the metabolic as well as synthetic activities during seed developmental and maturation determine

seed quality, which is expressed in seed germination and vigorous growth of seedlings. Tracing the pattern of development of seeds and its maturation behaviour is the main criteria for harvesting the crop at physiological maturity. Delaying in harvest reduces seed viability and vigour during storage. The stage at which the seed reaches its maximal dry weight and associated physical characteristics is referred as physiological maturity (Shaw and Loomis, 1950) [23]. Khattra and Singh, 1995[18] suggested that change in colour of pod or seed or both is the visual observation of physiological maturity.

In *vitro* method of propagation is majorly adopted for regeneration in medicinal plants, but seed propagation is highly multiplicative and economically viable. *Solanum trilobatum* L. is mainly propagated through seeds. Based on its high medicinal importance the plant needs to be conserved for future generation. By documenting the basic morphological traits, seed quality characters will help future researchers identify the crop's maturity trend. The evidence related to seed quality characters in nightshade were scanty. So, to fix the optimum stage of physiological maturity for proper harvest of seeds, the study was designed.

Materials and Methods

The experiment was conducted at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore during 2022-2023. The crop raised by the Department of Medicinal and Aromatic crops, Tamil Nadu Agricultural University, Coimbatore was utilized for the study. Sufficient number of fully opened flowers were tagged at five (5) days interval up to 40 days after anthesis (DAA) is designated as S_1, S_2, S_3, S_4, S_5, S_6, S_7 and S_8 which represents 5, 10, 15, 20, 25, 30, 35 and 40 days after anthesis. Four replicates of five berries were collected and the following observations were made for berry and seed characteristics. The berry characters such as colour, moisture content, fresh weight, dry weight, length, width, volume of berry and berry chlorophyll were recorded. The length, width of the berry has been measured using vernier caliper. The volume of berry was recorded by water displacement method using flat bottomed measuring cylinder (Kalavathi, 1996). The moisture content was estimated by hot air oven method (ISTA, 2015) [13]. The colour of berry and seed were observed by using Royal Horticultural Society (RHS) colour chart. Then the seeds were extracted manually from the berries collected from each developmental stage. The seed characters include fresh weight, dry weight, moisture content, number of seeds per berry and seed recovery were recorded.

The extracted seeds were subjected for germination using paper medium (Top of paper) and kept in germination room which is maintained at 25±2 ^{0}C and 95±2 per cent relative humidity (RH) to observe the physiological growth parameters. The berry and seed chlorophyll content has been analyzed as per Arnon (1949) [3].

Statistical analysis

The experiment was set up in a Completely Randomized Design (CRD) with four replications. The data were statistically analyzed using the procedure described by Gomez and Gomez, 1984 [9]. The critical difference (CD) was calculated at 5% (P = 0.05), and where the 'F' value is non-significant, it is represented by "NS". The Per cent values were converted into arc sine values wherever necessary.

Results and Discussion

Seed development and maturation is a genetically driven process which initiates from the ovule fertilization and ends up when the seed is independent from the parent plant. There are certain number of parameters such as moisture content and more dry weight accumulation helps to identify the maturation of seeds (Delouche, 1973) [7]. Physiological maturity is the stage of maximum dry weight accumulation and the nutrient supply from mother plant to seed terminates (Harrington, 1972) [12] by breakdown of vascular connection due to the formation of abscission layer. The main aim of tracing the pattern of seed development and maturation is to identify the physiological maturity which helps to harvest the crop at correct stage. Late harvest leads to loss of moisture, shattering of pods etc, whereas early stage of harvest leads to immature embryo. The pattern of seed development and maturation includes physical and physiological changes. The physical changes include increase in weight and change of colour in berries or pods. The high dry weight accumulation and germination of seeds comes under physiological changes (Vijay *et al*., 2020) [30].

Effect of maturation on berry characteristics during development and maturation

The present study was investigated with *Solanum trilobatum* L. to trace the pattern of seed development and maturation for harvesting high quality seeds. Highly significant results were observed on berry characters (Table 1). The fresh weight of berry was increased at each developmental stage. The maximum fresh weight (0.87 g) of berry were observed in 35 DAA and the weight starts to decrease at later stages. At initial stage of development, the berry observe more water which leads to increase in fresh weight at each developmental stage. The loss in fresh weight is mainly due to reduction of moisture content and also an escape of semi fluid volatiles along with water through the process of volatilization (Jayanthi *et al*., 2013) [15]. The diminishing pattern in fresh weight after 35 DAA could be attributed to berry dehydration and shrinkage similar results reported by Sundareswaran *et al.*, 2011 [27]. The dry weight of the berry at initial stage (5 DAA) is reported as 0.001 g and it is rapidly increased up to 0.84g in 35 DAA (Fig 4). The decrease in moisture content is caused by delayed respiration, resulting in significant dry weight accumulation (Rao *et al*., 1978) [22]. The maximum moisture content of berry (77.26%) was recorded at 15 DAA and it starts decreases rapidly up to 35 DAA (49.16%) it was evident with the report of McIIrath *et al*., 1963 [19], the water loss during maturation is regarded as an intrinsic phase of development. The moisture content can be reduced even after physiological maturation stage (Kalavathi, 1996) [16]. The initial berry volume has recorded as 15 DAA (0.43cm^3) which tend to increase rapidly up to 35 DAA (0.95cm^3). At 40 DAA the berry volume has decreased (0.83cm^3) due to loss of moisture and reduced weight of berry. Similar results have reported by Kalavathi (1996) [16] in Senna, Periwinkle and Roselle.

Table 1: Physical characters of berry during development and maturation in nightshade (*Solanum trilobatum* L.)

Stages of Berry development (DAA)	Berry length (mm)	Berry width (mm)	Berry volume (cm^3)	Fresh Weight (g)	Dry Weight (g)	Moisture content (%)	Berry Colour
S_1	0.00	0.00	0.00	0.002	0.001	0.00 (2.87)	Light green (RHS 149B)
S_2	8.81	9.66	0.00	0.20	0.19	0.00 (2.87)	Light green (RHS 144B)
S_3	9.24	10.07	0.43	0.34	0.32	77.26 (61.52)	Light green (RHS 144A)
S_4	9.87	10.58	0.63	0.60	0.59	66.14 (54.42)	Mild dark green (RHS 143A)
S_5	10.27	11.06	0.75	0.80	0.78	61.52 (51.66)	Mild dark green (RHS 141A)
S_6	11.03	11.22	0.93	0.81	0.79	52.66 (46.53)	Mild dark green (RHS 137A)
S_7	11.79	12.00	0.95	0.87	0.84	49.16 (44.52)	Mild red (RHS 168C)
S_8	11.40	11.55	0.83	0.83	0.82	46.37 (42.92)	Red (RHS 179A)
Mean	9.05	9.52	0.56	0.55	0.54	44.14 (41.63)	
S.Ed.	0.32	0.25	0.009	0.016	0.015	1.60	
CD (P 0.05)	0.68	0.52	0.020	0.033	0.032	3.30	

The berry length and width were observed maximum at 35 DAA (11.79 cm, 12.00 cm respectively), it represents the dimensions of berry has been increased significantly at each developmental stage. The berry chlorophyll was recorded maximum at 5 DAA (Fig 3) and it is reduced on later stages (Table 3). Damatta, 2008 [6] reported that, the plants are autotrophic which can easily generate food by photosynthesis (synthesis of light) process through the formation of chlorophyll. At early stage of development, the plant acquires more amount of sunlight to produce more amount of chlorophyll and it later stages the plants undergo senescence, the requirement of chlorophyll gets decreased due to less amount of generation of chlorophyll pigments. The chlorophyll in berry tends to decrease simultaneously with an advancement in maturation (Ward *et al*., 1995). The berry colour was initially light green (RHS 149B) at 5 DAA and it changes into mild red colour (RHS 168 C) at 35 DAA (Fig 1).

Effect of maturation on seed characteristics during development and maturation

The maximum moisture content of seed was observed at 15 DAA (55.94%) and it is reduced on further developmental stages due to rapid desiccation and dehydration (Abdul Baki-Anderson, 1973) [1]. Metha *et al*., (1993) [21] reported that, the decrease in moisture content with increased dry matter is clearly depicts the accumulation of food reserves in seeds (Fig 5). Loss of moisture content is due to maturation of seeds which indicates the supply of nutrient flow from mother plant to seed has terminated. The seed fresh weight and dry weight has been increased in each development stage (Table 2). The 100 seed weight were reached its maximum (0.28g) at 35 DAA. The seed recovery has initially recorded as (4.3%) at 15 DAA which were further increased up to (16.53%) at 35 DAA. Recovery rates varied by seed size but not by seed quantity or seed-size class proportions (Hagy, 2011) [11]. The number of seeds per berry has been recorded as 18 at 15 DAA and it is increased up to 35 at 35 DAA. The difference in the number of seeds per berry was caused by variation in berry size and sparsely filled seeds during seed development. It is directly proportional to the size and weight of the flower. Seed germination is the prime indicator of seed quality. The germination potential of seed represents the planting value of seed. The seeds become dormant during maturation phase until they shed from the mother plant or to meet out the certain germination criteria (Samarah *et al*., 2004) [23].

The seed crop *Solanum trilobatum* L. has recorded nil germination at each developmental stage which could be due to the presence of dormancy, prevents the germination. Similar results were reported by Jayamani (2020) [14] in Black cumin (*Nigella sativa*).

Table 2: Physical and physiological characters of seed during development and maturation in Nightshade (*Solanum trilobatum* L.)

Stages of Seed development (DAA)	Fresh weight (g)	Dry weight (g)	100 Seed Weight (g)	No. of. seeds/berry	Moisture content (%)	Seed recovery (%)	Seed germination (%)	Seed colour
S_1	0.00	0.00	0.00	0	0.00 (2.87)	0.00 (2.87)	0 (2.87)	-
S_2	0.00	0.00	0.00	0	0.00 (2.87)	0.00 (2.87)	0 (2.87)	Yellow (RHS 5C)
S_3	0.02	0.01	0.04	18	55.94 (48.41)	4.33 (12.00)	0 (2.87)	Mild yellow (RHS 7D)
S_4	0.08	0.05	0.09	30	49.14 (44.51)	8.33 (16.77)	0 (2.87)	Mild yellow (RHS 8B)
S_5	0.09	0.07	0.16	31	36.94 (37.43)	8.88 (17.33)	0 (2.87)	Mild yellow (RHS 10B)
S_6	0.13	0.09	0.21	35	24.40 (29.60)	15.45 (23.15)	0 (2.87)	Mild yellow (RHS 11B)
S_7	0.17	0.14	0.28	35	15.79 (23.41)	16.53 (23.99)	0 (2.87)	Yellowish white (RHS 11C)
S_8	0.15	0.12	0.25	35	14.37 (22.27)	15.81 (23.43)	0 (2.87)	Yellowish white (RHS 4D)
Mean	0.08	0.06	0.12	23.0	24.57 (29.71)	8.66 (17.11)	0 (2.87)	-
S.Ed.	0.01	0.01	0.01	6.29	1.22	2.21	-	-
CD (P 0.05)	0.02	0.02	0.03	12.99	2.53	4.56	-	-

Table 3: Biochemical characters of berry during development and maturation in Nightshade (*Solanum trilobatum* L.)

Stages of Berry development (DAA)	Berry		
	Chlorophyll A	Chlorophyll B	Total chlorophyll
S_1	1.07	0.80	1.870
S_2	0.90	0.73	1.623
S_3	0.79	0.62	1.418
S_4	0.68	0.56	1.246

S_5	0.59	0.48	1.073
S_6	0.52	0.37	0.895
S_7	0.45	0.31	0.763
S_8	0.37	0.26	0.628
Mean	0.67	0.51	1.18
S.Ed	0.03	0.02	0.05
CD (P 0.05)	0.08	0.05	0.12

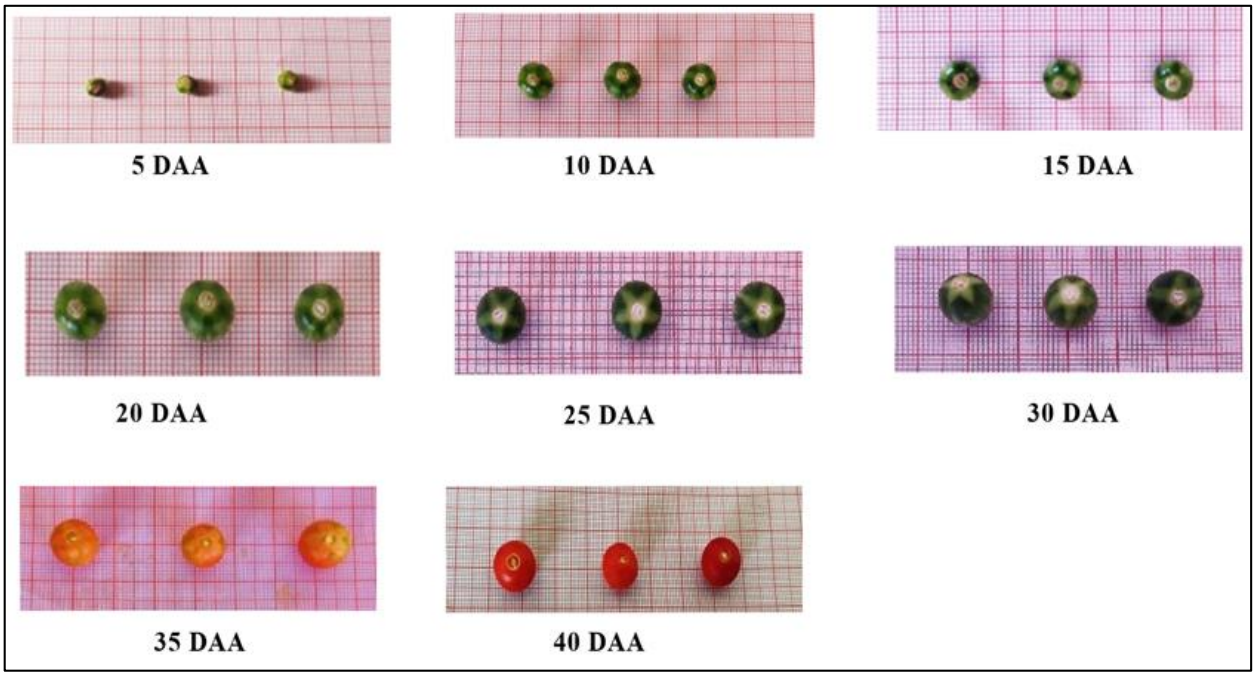

Fig 1: Development of berries at different stages in nightshade (*Solanum trilobatum* L.)

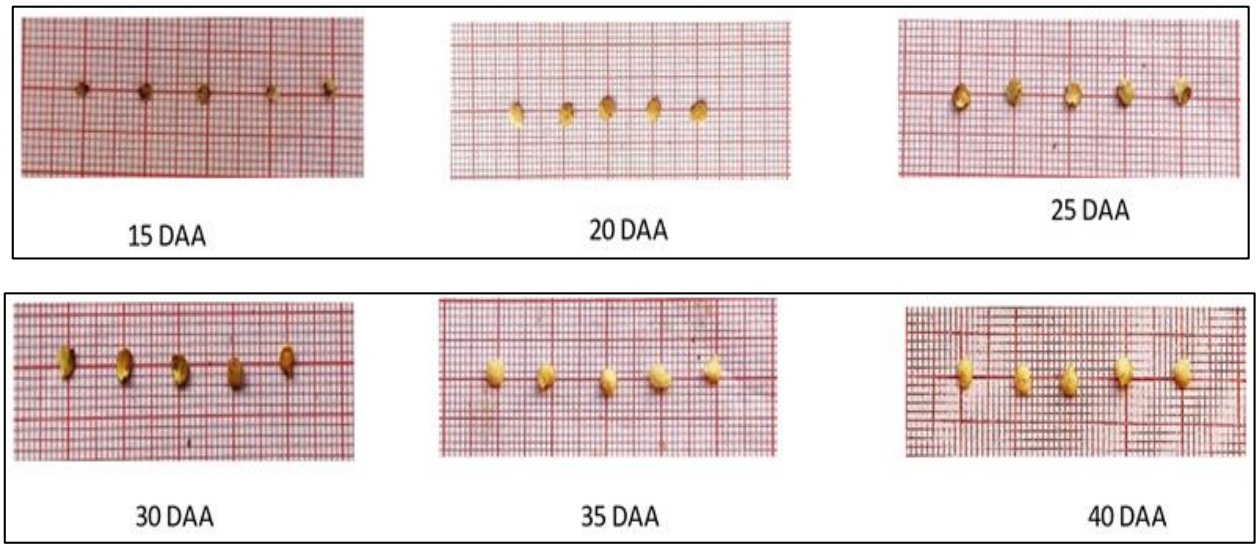

Fig 2: Development of seed at different stages in nightshade (*Solanum trilobatum* L.)

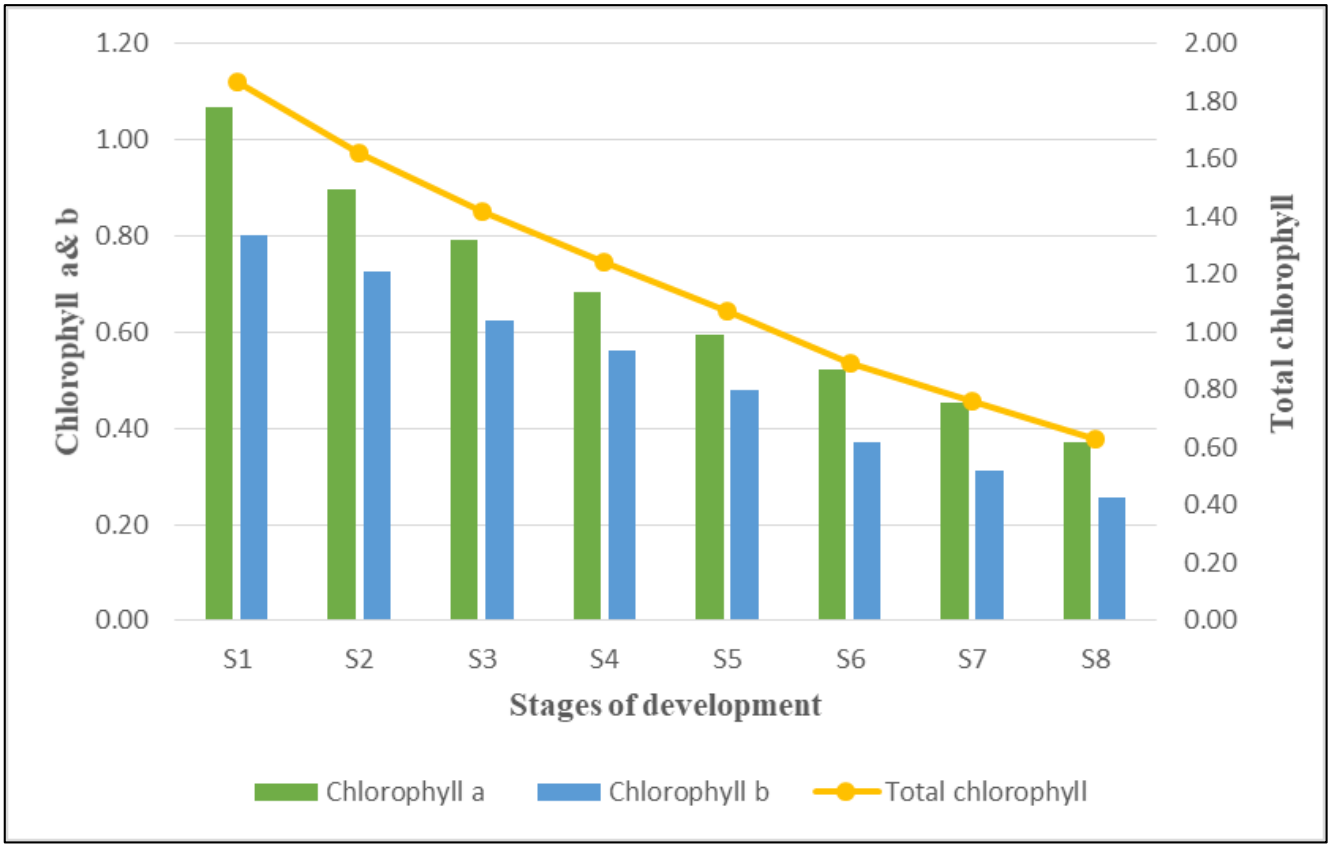

Fig 3: Changes in berry chlorophyll during development and maturation in Nightshade (*Solanum trilobatum* L.)

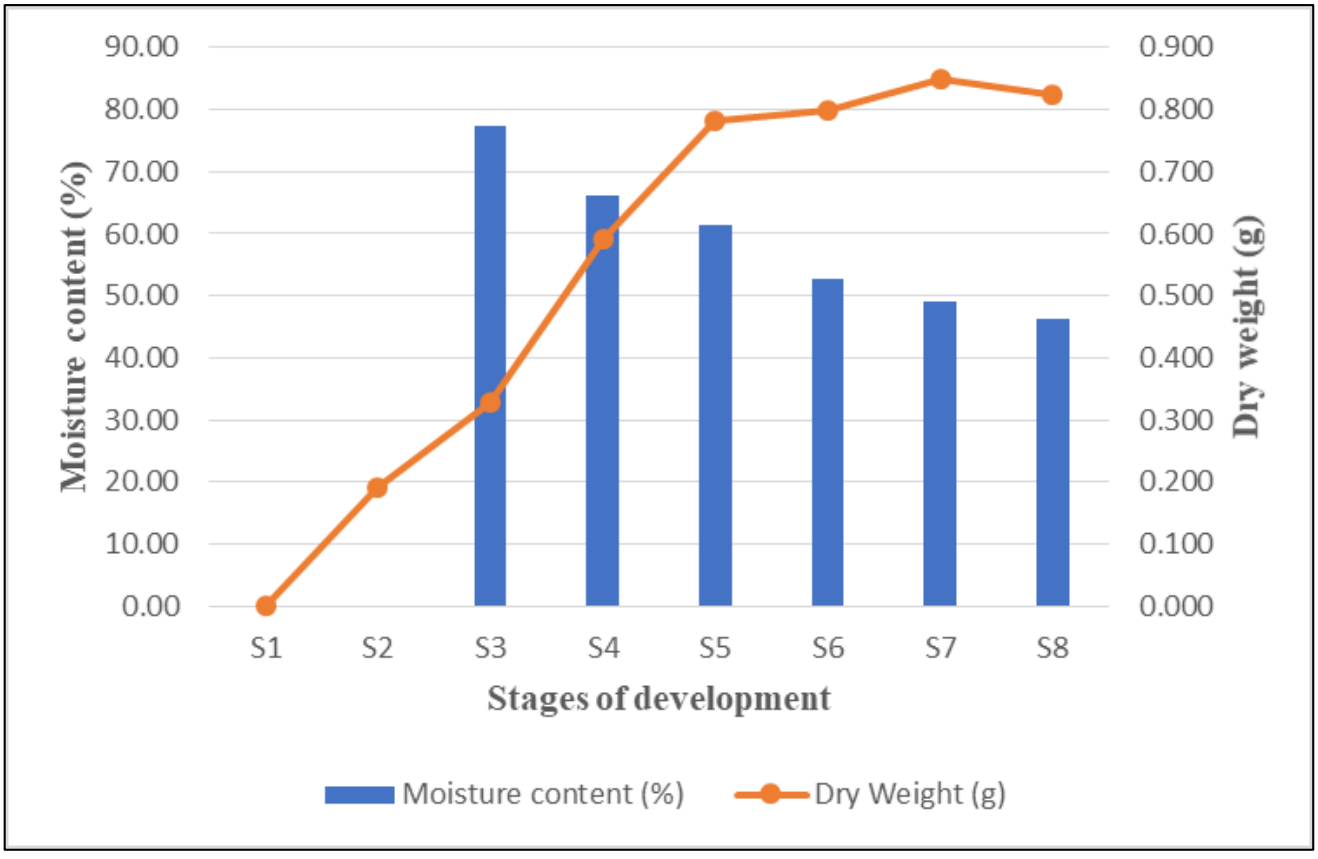

Fig 4: Changes in moisture content and dry weight of berry during maturity stages of Nightshade (*Solanum trilobatum* L.)

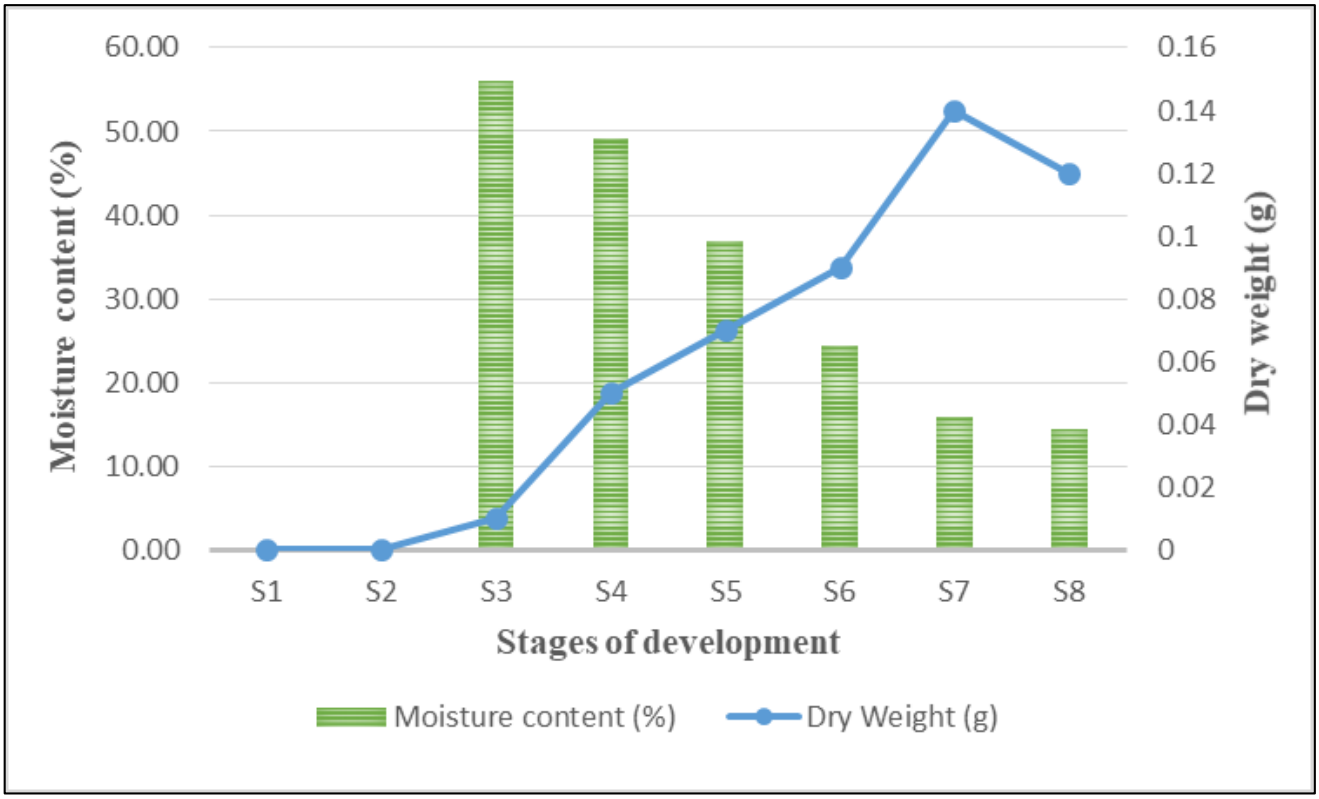

Fig 5: Changes in moisture content and dry weight of seed during maturity stages of Nightshade (*Solanum trilobatum* L.)

Conclusion

The study on tracing the pattern of seed development and maturation in nightshade revealed that the berry attained physiological maturity at 35 DAA, which can be visually observed by change in colour of berry from light green to mild red. The result coincides with the maximum accumulation of dry matter in berry 0.84g with minimum moisture content 49.16% and 16.53% of seed recovery. Similarly, the seed attained its physiological maturity on 35 DAA with high dry matter accumulation 0.14g with minimum moisture 15.79%.

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