International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2020; 8(5): 317-322

Received: 11-06-2020
Accepted: 20-07-2020

M Saranya
Post graduate research scholar, Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Dr. J Renugadevi
Professor, Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Dr. K Raja
Assistant professor, Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Dr. S Marimuthu
Assistant professor, Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Dr. V Babu Rajendra Prasad
Assistant professor, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
M Saranya
Post graduate research scholar, Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Nanoemulsion seed coating for heat stress management in soybean

M Saranya, J Renugadevi, K Raja, S Marimuthu and V Babu Rajendra Prasad

DOI: https://doi.org/10.22271/chemi.2020.v8.i5e.10313

Abstract
An experiment was conducted to access the effect of methyl cellulose nanoemulsion seed coating in soybean (JS-93-05) against heat stress. The nanoemulsion was prepared by using methyl cellulose, vitamin E and tween 80 (in the form of oil in water) with the help of high energy homogenizer. The properties of nanoemulsion were characterised by using Particle size analyzer. (PSA), Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). Soybean seeds e coated with nanoemulsion @ 20ml/kg and kept in plant growth chamber where the temperature and relative humidity were maintained at 40^0C and 50% for a duration of 0, 3, 5, 7, 9, 11 and 13 days and simultaneously seed quality was determined. The results demonstrate that nanoemulsion coated seeds recorded higher rate of germination, speed of emergence, seedling length, vigour index and root volume compared to uncoated seeds under high temperature stress condition.

Keywords: Soybean, nanoemulsion, encapsulation, temperature stress, enhanced vigour

Introduction

Soybean (*Glycine max* L.) is the most important legume cum oil seed crop, which contributes to 25 % of the global edible oil, about two-thirds of the world's protein concentrate for livestock feeding. The contribution of India in soybean cultivation area is 10 % (11 mha) in the world with the production of 9 million metric tons and the productivity of 0.80 metric tons/ha. In India, soybean is predominantly grown as a rainfed crop, covering the states of Madhya Pradesh, Maharashtra and Rajasthan. In Peninsular India, soybean grown under rainfed agro-ecosystem. Rainfed agriculture continuously plays a major role in food availability and economic status in India. The success of the rainfed agriculture depends upon the maintenance of vigour and viability of seeds till the seeds received optimum soil moisture for emergence and establishment (Surendhiran *et al.*, 2019) [13]. The crop growth and yield is based on the improved seed germination and production of healthy seedling. For improving the seed potential under abiotic stress condition, various seed treating methods are used such as seed coating, priming, pelleting, hardening and soaking the seeds in growth regulators, various organic and inorganic materials (Sakthivel *et al.*, 2016) [11]. Adoption of nanomaterial seed treatment is the newly emerging technology for enhancing the seed quality. Seeds coated with carboxyl methyl cellulose based IAA nanoemulsion recorded the better performance compared to control seeds in groundnut (Tamilarasan *et al.*, 2018) and cotton (Sakthivel *et al.*, 2016) [11]. Considering the significance of nano seed invigouration, the study was conducted to find out the effect of nanoemulsion coating in soybean for mitigation of high soil temperature stress.

Materials and methods

The experiment was conducted at Department of Seed Science & Technology, Department of Nano Science & Technology and Department of Crop Physiology in Tamil Nadu Agricultural University, Coimbatore during 2019-2020. Soybean seeds (JS-93-05) were obtained from Indian Institute of Soybean Research, Indore, Madhya Pradesh, India. Methyl cellulose, Tween 80 and vitamin E were obtained from M/s. Sigma Aldrich Chemicals Private Limited, Bangalore, India formed the base materials for the study.

i) Synthesis and characterization of methyl cellulose nanoemulsion

A. Preparation of 2% methyl cellulose stock solution Methyl cellulose (2 percent) solution was synthesized by dissolving 2g methyl cellulose in 100 ml of distilled water and kept in continuous stirring in magnetic stirrer at the level of 450 rpm until the formation a clear solution.

B. Vitamin E stock solution Vitamin E (1 percent) solution was prepared by dissolving 1g of vitamin E in 100 ml of absolute ethanol or alcohol.

Preparation of conventional emulsion

Conventional methyl cellulose emulsion (100ml) was prepared by using methyl cellulose, vitamin E and Tween 80 in the ratio of 99.4: 0.1: 0.5, and kept for constant stirring in magnetic stirrer at 450 rpm.

Preparation of methyl cellulose nanoemulsion using high energy homogenizer

The conventional emulsion was subjected to high energy homogenization at 40000 bar (Sharma *et al.*, 2015) [12].

Characterization of nanoemulsion

Nanoemulsion was analysed for particle size, Scanning Electron Microscope (SEM) Transmission Electron Microscope (TEM)

The Particle Size Analzer: Particle size and distribution pattern of methyl cellulose nanoemulsion were determined using Nanopartica SZ-100, Horiba Scientific. Five ml of sonicated nanoemulsion was taken and analyzed under dynamic light scattering method using 90º at 25ºC.

Scanning electron microscope (SEM) and transmission electron microscope (TEM)

Morphological characteristics of nanoemulsion sample were analysed by Scanning Electron Microscope imaging (Quanta 250, FEI) and Transmission Electron Microscope (TEM FEI TECHNAI SPRIT) available at Department of Nano Science and Technology, TNAU, Coimbatore.

Scanning electron microscope (SEM)

Methyl cellulose nanoemulsion was kept over the aluminium stub and loaded in the sample holder. Sample surface was observed at different magnifications and the images were recorded.

Transmission electron microscope (TEM)

Nanoemulsion was taken after sonication process for 15 min. and placed on the copper grid and allowed to dry in vacuum for 24 hrs. Then the copper grid was placed on the sample holder and the images were viewed at different magnifications.

Effect of nanoemulsion seed coating on seed quality under temperature stress conditions

Soybean seeds were coated with methyl cellulose nanoemulsion at the concentration of 20ml/kg of seed. The coated seeds were exposed to high temperature with the use of plant growth chamber where the temperature and relative humidity were maintained at 40^0C and 50% respectively for the duration of 3, 5, 7, 9, 11 and 13 days. The performance of coated seeds along with uncoated seeds after the exposure to high temperature stress were evaluated for the seed quality characters by recording the various observations such as speed of emergence (Maguire,1962) [7], germination root length, shoot length, vigour index and root volume. The statistical method used for this experiment was factorial completely randomized design (FCRD) with four replications and analysed using OPSTAT.

Speed of germination (Maguire, 1962) [7]

Four replications of 100 seeds of uncoated and coated seeds were taken after high temperature exposure of various days (0, 3, 5, 7, 9, 11, 13 days exposed seeds) and seeds were kept in pot culture medium which contains field soil. The germinated seedling from pot were counted regularly from first day to 7^{th} day.

Based on the number of seeds germinated on each day, the speed of germination was calculated using the following formula and the result was expressed in number.

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots + \frac{X_n - X_{n-1}}{Y_n}$$

Where, X_1 Seeds germinated during first count (No's)
X_2- Seeds germinated during second count (No's)
X_n- Seeds germinated during n^{th} day (No's)
Y_1- Number of days from sowing to first count
Y_2- Number of days from sowing to second count
Y_n- Number of days from sowing to n^{th} count

Germination percentage

Germination test was conducted by using 100 seeds in 4 replications. The pot size was 15 × 15 cm. At the end of 7^{th} day of germination, the number of normal seedlings was counted and the mean data value was expressed as percentage.

Root length

Ten normal seedlings were randomly (equally distributed) collected from the germination test. Root length was observed from collar region to the tip region of the root. The mean value was expressed in centimeter.

Shoot length

The seedlings which were used in root length measurement were again used for measuring the shoot length. The mean value of shoot length was expressed in centimeter.

Dry matter production

Ten seedlings which were used in above measurements were initially shade dried for 24 h. After that it were dried again in oven for 24 hrs which was maintained at 85°C and then cooled in a desiccators (silica gel) for 30 mins. The dried seedlings were weighed on weighing balance, and the mean value expressed in mg 10 seedlings^{-1}.

Vigour index (Abdul-Baki and Anderson, 1974) [1]

Based on the germination, total seedling length and dry matter production, the vigour index I and II were calculated by using the following formula and the value was expressed in whole number.

Vigour index I = Germination percentage × Seedling length
Vigour index II = Germination percentage × Dry matter production

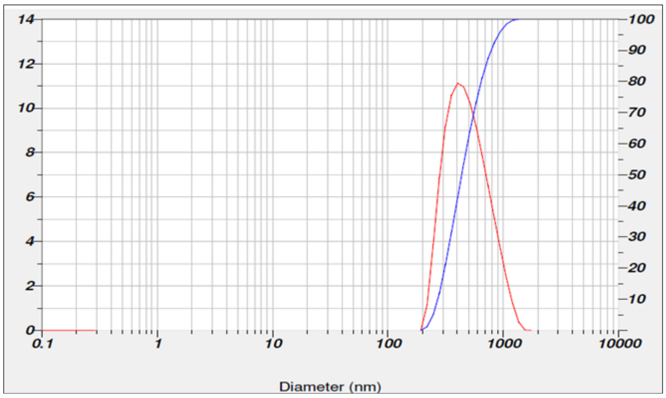
Root volume

The seedlings used in root length were again used for measuring the root volume. The roots were dipped in the measuring cylinder containing particular amount of water, it considered as a initial value. After dipping of roots, the amount of water level were increased. It considered as a final value). The root volume were measured by formula and the values were expressed in cc.

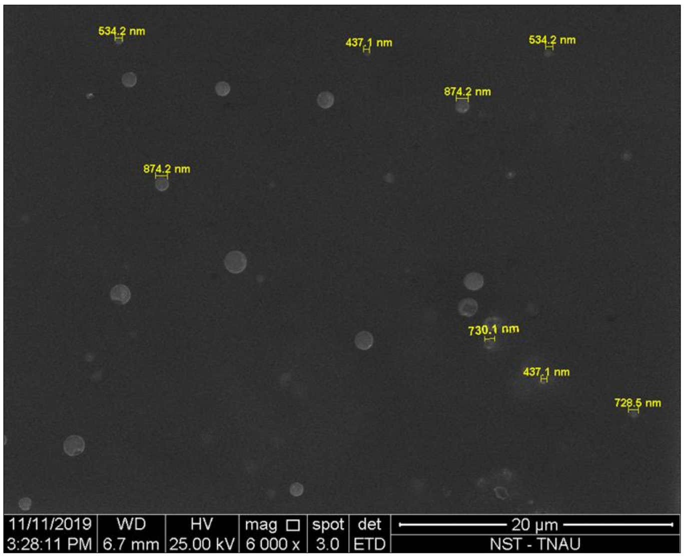
Root volume = Final value – Initial value

Result and discussion

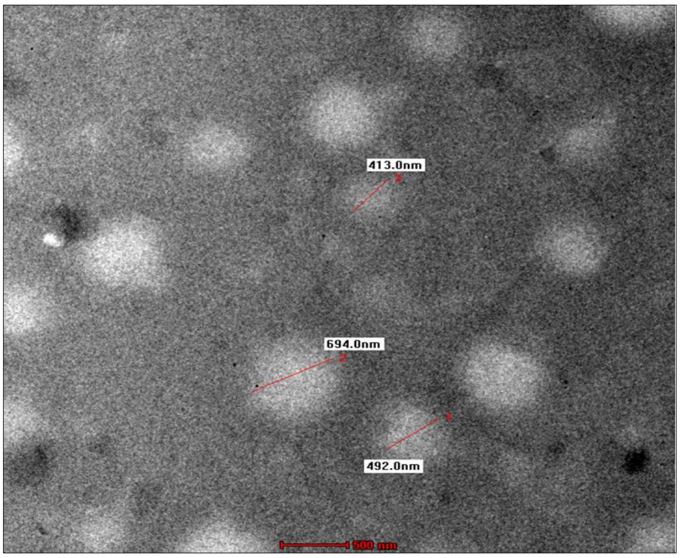
Methyl cellulose nanoemulsion prepared by high energy homogenizer method at 40000 bars revealed that the particle size of 565.4 nm (Figure 1). The SEM images revealed the core shell structured particles ranges from from 400 to 900nm, further supported from images of TEM which give the core shell structure of particles sized from 300 to 800 nm



Picture 1: Particle size analysis of nanoemulsion



Picture 2: SEM images of nanoemulsion



Picture 3: TEM images of nanoemulsion

Picture 4: 9 days temperature exposed soybean seedlings

(Figure 2 and 3).The droplet size of the emulsion was based upon the homogenization pressure. In this present study also, increase in homogenization pressure leads to the reduction in particle size. Hence that homogenization at 40000 bar produced the smallest particle size in emulsion.

Constant and continuous stirring results in the formation of fine droplets due to quick diffusion of surfactant molecule from the organic phase into the aqueous phase (Tamilarasan *et al*., 2018). The reduced particle size at high energy level is due to rupturing of particles where the shear forces acting upon emulsion droplets which leads collision and disrupts the interfacial membrane. The particle size reduction might be also due increasing the homogenization cycle that declines the surface tension, which leads to increase in rate of surfactant adsorption and reducing the ratio of viscosity (Jasmina *et al.*, 2017) [4]. The fine particle size with excellent stability may be due to the molecular structure of Tween 80 wherein the presence of hydrophobic chain in tail (C18 = 1, a mono oleate tail) induces the mobility of particles that prevents the agglomeration (Hasani *et al.*, 2015) [2]. The surface topography of nanoemulsion analysed under SEM reported that methyl cellulose nanoemulsion had a core shell shaped particles with size ranges from 400 to 900nm. Further, the results of TEM images confirmed that the morphology of nanoemulsion as core shell structure with size ranges from 300 to 800 nm. The polymer nanoemulsion prepared by using carboxy mehyl cellulose exhibited the core shell like morphology of the particles under SEM and TEM analysis (Tamilarasan *et al.*, 2018). Surendhiran *et al.* (2019) [13] reported that methyl cellulose nanoemulsion prepared at high energy homogenization had the required particle size and core shell structure morpholohy of micelle. Sakthivel (2016) [11] reported the core shell like structure of micelle observed in cellulose based hormones incorporated nanoemulsion.

Effect of nanoemulsion seed coating in soybean under high temperature stress condition

The results of speed of emergence had a significant difference in coated seeds compared to uncoated seeds. Speed of emergence was reduced in coated and uncoated seeds at the temperature exposure period extended from 0 day (7.53) to 13 days (5.51). However the coated seeds recorded a maximum speed of emergence than uncoated seeds.

Nanoemulsion seed coating had momentous difference in recording germination (%) of coated seeds compared with uncoated seeds. Similar to that of other results, germination percentage was reduced in 13 days exposed seeds (55%) compared to 0 day exposed seeds (89%) of both coated and uncoated seeds. The maximum germination (%) was recorded by unexposed coated soybean seeds with (90%) and minimum was recorded by uncoated seeds of 13 days exposure (50%). Results of root length revealed that the influence of nanoemulsion on seedling length (Picture 4). Depending upon the temperature exposure period, root length was reduced in 13 days exposed seeds (14.1cm) compared to 0 day exposed seeds (18.7cm) in both coated and uncoated seeds. Treated seeds of soybean with 0 day exposure recorded the maximum root length (19.1 cm) and the uncoated seeds of soybean with 13 days exposure recorded the minimum root length (13.1 cm).

Shoot length showed the significant difference due to nanoemulsion coating. Irrespective of treatments, shoot length was reduced based on the temperature exposure period in both coated and uncoated seeds from 0 day exposure (15.0cm) to 13 days exposure (8.8cm). Dry matter production was significantly influenced by the seed coating. Depending upon temperature exposure period, dry matter production reduced in 13 days exposed seeds (1.05 g 10 seedlings^{-1}) compared to 0 day exposed seeds (1.51g 10 seedlings^{-1}) in both coated and uncoated seeds. The highest dry matter production was recorded by the coated seeds of 0 day exposure (1.55 g 10 seedlings^{-1}) and the lowest by the uncoated seeds of 13 days exposure (0.99 g 10 seedlings^{-1}).

Vigour index of coated seeds significantly differed from uncoated seeds. Increase in duration of temperature exposure period reduced the VI-I in both coated and uncoated seeds from 3005 (0 day exposure) to 1272 (13 days exposure). Vigour index-I was maximum in coated seeds of 0 day exposure (3151) and minimum in uncoated seeds of 13 days exposure (1070). Vigour index-II was reduced in both coated and uncoated seeds due to high temperature exposure from 134 (0 day exposed) to 61 (13 days exposed).Vigour index-II was maximum in 0 day exposed coated seeds. Root volume was reduced from 7.35cc (0 day exposure) to 6.50cc (13 days exposure) in both coated and uncoated seeds based on temperature exposure period. Maximum root volume was observed in coated seeds with 0 day exposure (7.6 cc) and minimum for the uncoated seeds with 13 days exposure (5.5 cc).

Jain *et al.*, 2013 reported that cellulose derivative polymers are the linear homopolymer polysaccharide and having water insoluble property. Thermoresponsive polymers are sensitive to thermal environment surrounding them and in response to it they show change in their property. In this present study, nano polymer used is a cellulose derivative (methyl cellulose nanoemulsion) had a thermal protective nature under high temperature stress condition and give protection for seeds to withstand high soil temperature condition

The effect of polymer coating on seed quality improvement was reported by several scientists. Taylor *et al.*, 2001 [16] reported that application of the polymer on the seeds acts as exterior or covering shell. It modifies the water uptake and enhances the seed germination and leads to better emergence and establishment of seedlings.

The positive effect of nanoemulsion seed invigouration on germination and seedling vigour of soybean seed under high temperature attributed to phase transition behaviour of methyl cellulose which forms a film on seed coating and acts as thermal barrier that protects the seeds against high temperature stress and when the polymer reaches the ambient temperature it changes its structure and allow the water for facilitating the seeds to imbibe and germinate.

Methyl cellulose form films including different additives (alpha tocopherol) for biodegradability and antioxidative properties which become an excellent barrier against ultra violet radiation and high temperature (Pauline *et al.*, 2015) [9]. According to Kumar *et al.* (2007) [6] methyl cellulose could be used as potential seed coating agent for protecting the seeds against abiotic stress and he observed that the soybean seeds coated with methyl cellulose had significantly recorded higher germination and seedling vigour as compared to uncoated seeds. In the present investigation, higher seedling vigour and biomass is due to the maximum root and shoot growth. Nasima *et al.* (2010) [8] reported that maize seeds coated with biodegradable polymer at 2 percent concentration registered maximum germination and seedling vigour than uncoated seeds Sakthivel *et al.* (2016) [11] in cotton, CMC based nanoemulsion fortified with GA_3 and IAA recorded the improved germination, vigour potential and seedling length under controlled condition. CMC based nanoemulsion which contain GA_3 and IAA recorded improved germination of 10 percent over control seeds. Similarly the effect of methyl cellulose nanoemulsion coating in soybean seeds under high temperature stress condition demonstrated that coated seeds of soybean recorded the higher seed quality parameters such as germination, root length, shoot length, speed of emergence, dry matter production and root volume after the exposure to high temperature for a period of 0, 3, 5, 7, 9, 11 and 13 days. The naoemulsion coated seeds recorded 20 percent improved germination over control even

Table 1: Effect of nanoemulsion seed coating on physiological parameters of soybean (JS-93-05)

Duration of exposure to high temperature (40^0C) Days(D)	Treatment(T)											
	Speed of emergence			Germination (%)			Root length(cm)			Shoot length(cm)		
	T_0	T_1	Mean	T_0	T_1	Mean	T_0	T_1	Mean	T_0	T_1	Mean
0	7.15	7.91	7.53	88	90	89	18.3	19.1	18.7	14.1	15.9	15.0
3	6.79	7.49	7.14	80	88	84	16.8	18.3	17.5	11.7	14.8	13.2
5	6.57	7.18	6.87	76	84	80	16.1	18.0	17.1	10.7	14.2	12.5
7	6.51	6.95	6.73	74	78	76	15.7	17.7	16.7	9.8	11.5	10.71
9	6.49	6.64	6.56	65	75	70	15.2	17.1	16.2	9.6	10.8	10.27
11	5.38	6.19	5.78	55	65	60	14.1	16.2	15.2	8.9	9.7	9.35
13	5.19	5.83	5.51	50	60	55	13.1	15.1	14.1	8.2	9.4	8.84
Mean	6.30	6.88	6.59	69	77	73	15.6	17.4	16.5	10.4	12.3	11.39
	T	D	TXD	T	D	TXD	T	D	TXD	T	D	TXD
SE(d)	0.03	0.06	0.08	0.91	1.70	2.42	0.12	0.22	0.32	0.16	0.31	0.44
CD	0.06	0.12	0.16	1.84	3.45	NS	0.29	0.45	NS	0.34	0.63	0.90

Table 2: Effect of nanoemulsion seed coating on physiological parameters of soybean (JS-93-05)

Duration of exposure to high temperature (40^0C) Days(D)	Treatment(T)											
	Dry matter production(g/10 seedlings)			Vigour index I			Vigour index II			Root volume (cc)		
	T_0	T_1	Mean	T_0	T_1	Mean	T_0	T_1	Mean	T_0	T_1	Mean
0	1.47	1.55	1.51	2860	3151	3005	129	139	134	7.1	7.6	7.35
3	1.25	1.48	1.36	2288	2914	2601	100	130	115	6.5	7.2	6.85
5	1.21	1.42	1.31	2044	2713	2378	91	119	105	6.4	7.0	6.70
7	1.12	1.38	1.25	1897	2285	2091	82	107	95	6.2	6.7	6.45
9	1.08	1.32	1.20	1620	2105	1862	70	99	85	6	6.6	6.30
11	1.03	1.28	1.15	1269	1693	1481	56	83	70	5.7	6.3	6.00
13	0.99	1.12	1.05	1070	1475	1272	49	73	61	5.5	6.1	5.80
Mean	1.16	1.36	1.26	1864	2333	2098	82	107	95	6.2	6.8	6.50
	T	D	TXD	T	D	TXD	T	D	TXD	T	D	TXD
SE(d)	0.03	0.05	0.08	11.12	20.82	29.44	1.09	2.04	2.89	0.08	0.15	0.21
CD	0.06	0.11	NS	22.53	42.16	59.63	2.22	4.14	5.86	0.16	0.30	NS

After 13 days of temperature exposure. The results showed that the impact of nanoemulsion on seed quality improvement and production of potential seedlings in soybean.

Dry matter production of the coated seedlings recorded the percentage increase of 5.4 percent over the uncoated seeds even in 0 day exposure. Similarly 13 days after exposure, coated seeds recorded the increased dry matter production of 13.1 % over uncoated seeds. Nanoemulsion coated seeds had the improved physiological activities. Tamilarasan *et al.* (2018) stated that the effect of CMC fortified with growth regulators (GA_3 and IAA) on seed quality improvement. IAA loaded nanoemulsion at the rate of 15ml/kg recorded the improved physical and physiological activities at optimal and suboptimal conditions in different seed lots in groundnut.

In the present study, the performance of nanoemulsion coated seeds was found to be superior than the uncoated seeds, since the polymer used to prepare the emulsion was methyl cellulose which is hydrophilic in nature. The emulsion coated seeds imbibed the water quickly compared to uncoated seed and nanoemulsion acts as outer shell and protects the seed from high temperature and reduces the moisture loss from seeds under high temperature stress condition. Similarly the other physiological activities were triggered, resulted in early seedling establishment. This was supported by Surendhiran *et al.* (2019) [13] who reported that maize seeds coated with methyl cellulose based emulsion recorded the high germination, seedling length, vigour index, imbibition rate and dry matter production.

Conclusion

The result of the present temperature study clearly reviewed that methyl cellulose nanoemulsion coated soybean seeds recorded the better seedling quality performance compared to the uncoated seeds under high temperature stress condition. The soybean seeds exposed to high temperature stress for 9 days recorded the germination percentage of 65% (below IMSCS) and the seeds coated with methyl cellulose nanoemulsion maintained 75% of germination even after 9 days of temperature exposure. Thus the result clearly demonstrated that, methyl cellulose nanoemulsion coated seeds of soybean had the ability to withstand the high temperature stress without reduction in seed quality. Hence, the methyl cellulose nanoemulsion seed coating @ 20ml/kg of seeds could be recommended for the enhancement seed vigour and seedling establishment for pre-monsoon sowing.

References

1. Abdul-Baki A, Anderson JD. Vigor determination in soybean seed by multiple criteria. Crop Science. 1974; 13:630-633.
2. Hasani F, Pezeshki A, Hamishehkar H. Effect of Surfactant and Oil Type on Size Droplets of Betacarotene-Bearing Nanoemulsions. International journal of Current Microbiology and Applied Sciences. 2015; 4(9):146-155.
3. ISTA. International Rules for Seed Testing. The International Seed Testing Association, Bassersdorf, Switzerland, 2013.
4. Jasmina H, Džana O, Alisa E, Edina V, Ognjenka R. Preparation of nanoemulsions by high-energy and lowenergy emulsification methods. In CMBEBIH. 2017; 317-322. Springer.
5. Jain S, Sandhu PS, Gupta B. Cellulose Derivatives as Thermo responsive Polymer: An overview. Journal of Applied Pharmaceutical Science. 2013; 3(12):139-144.
6. Kumar J, Nisar K, Arun Kumar MB, Walia S, Shakil NA, Prasad R *et al.* Development of polymeric seed coats for seed quality enhancement of soybean (Glycine max).

Indian journal of agricultural science. 2007; 77(11):738-743.
7. Maguire JD. Speed of germination in selection evaluating for seedling emergence and vigour. Crop Sciences. 1962; 2:176-177.
8. Nasima J, Khanif MY, Hanfi MM, Wan ZWY, Dharejo KA. Maize response to biodegradable polymer and urease inhibitor coated urea. International Journal of Agriculture and Biology. 2010; 12(5):773-776.
9. Paulín EG, MSP MME, Lara-Sagahón AV, Torres Pacheco I. Maize seed coatings and seedling sprayings with chitosan and hydrogen peroxide: their influence on some phenological and biochemical behaviors. Journal of Zhejiang University Science. 2015; 14(2):87-96.
10. Raja K, Sowmya R, Sudhagar R, Moorthy PS, Govindaraju K, Subramanian KS. Biogenic ZnO and Cu nanoparticles to improve seed germination quality in blackgram (*Vigna mungo*). Materials Letters. 2019; 235:164-167.
11. Sakthivel. Development of hormone loaded nanoformulations (nanoemulsions) for improving the germination and vigour of cotton M. Sc, Seed Science & Technology Tamil Nadu Agricultural University, 2016.
12. Sharma T, Velmurugan N, Patel P, Chon BH, Sangwai JS. Use of oil-in-water pickering emulsion stabilized by nanoparticles in combination with polymer flood for enhanced oil recovery. Petroleum Science and Technology. 2015; 33(17-18):1595-1604.
13. Surendhiran M, Raja K, Jerlin R, Marimuthu S, Srivignesh S. Nano emulsion seed invigouration for improved germination and seedling vigour in maize. International Journal of Agricultural Science and Research (IJASR). 2019; 9(3):333-340.
14. Tamilarasan C, Raja K, Subramanian S, Selvaraju P. Synthsis and development of nano formulation for hastening seed quality in Groundnut. Res. Jr. of Agril. Sci. 2019; 10(1):50-57.
15. Tamilarasan. Studies on the efficacy of hormones loaded nanoformulation on seed quality of groundnut (*Arachis hypogaea* L.). M. Sc(Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, 2017.
16. Taylor A, Kwaitowski J. Polymer film coatings decrease water uptake and water vapour movement into seeds and reduce imbibitional chilling injury. Bcpc Symposium Proceedings. 2001; 2:21-24.