Pesticide Research Journal Vol. 24(1): 55-64, June 2012

Quality Enhancement of Soybean Seed Coated with Nano-formulated Thiamethoxam and its Retention Study

Dhruba Jyoti Sarkar, Jitendra Kumar*, NA Shakil and S Walia
Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi 110 012, India

The use of insecticides for seed treatment is one of the most effective modern pest control practices. Therefore, the influence of these products on the physiological quality of treated seeds should be known. This study aimed to evaluate the physiological quality of thiamethoxam treated soybean seeds stored for three different periods. Seed coating was performed with 12 controlled release (CR) formulations of thiamethoxam based on amphiphilic polymers along with commercial formulation and control. The percent thiamethoxam recovery ranged from 86.1-93.2% from different CR formulation seed coats. Better thiamethoxam retention was observed on soybean seed coats treated with CR formulations. Different seed quality parameters *viz*., seed germination, seed vigour, seed moisture and seed storability was evaluated during the six month experimentation. The seeds coated with different nano-formulations had better seed quality over control and commercial formulation.

Key words: Controlled release, amphiphilic polymers, thiamethoxam, retention, seed germination, seed vigour

Seed quality comprises several physical, chemical and biological components. Seed being a biological or living entity, deterioration in its quality with time is inevitable. However, the rate of deterioration could be slowed down either by storing seeds under controlled conditions or by imposing certain treatments with either chemicals or other protectant. As the controlled environment involves huge cost, the seed treatment remains the best alternative approach to maintain seed quality. The most common seed treatments alleviate biotic stress by reducing the damage caused by seed or soil borne pests and pathogens (e.g., insects and fungi) on seeds and seedlings[1-3].

The term “coated seed” has been applied to a seed, which was either pelleted, coated or covered with an adhesive film. Coated seed may, in some circumstances, be produced by a dry powder process, which can have several disadvantages, such as poor adherence, non-uniform application, generation of significant amounts of dust, etc.[4,5]. Seed coating with polymers is one such pre-storage treatment that can be used either singly or in combination with other pesticides as formulation to protect seeds against a pests and diseases. The possibilities of using polymers along with other chemicals to ensure the keeping quality of seeds are reported[6]. The main advantage of this technology includes better adherence of active ingredients, uniform coating and improved seed flow through planter. Coating with a hydrophilic polymer has been reported to regulate the rate of water uptake, reduce imbibitional damage and improve the emergence of soybean seeds[7]. Maize seed coating was performed with temperature responsive polymer to regulate seed germination [8]. The effects of polymer coating on seed vigour in rice have also been reported[9]. In this work, the effect of nano sized controlled release seed coat formulations of thiamethoxam based on amphiphilic polymers is reported on germination percentage, weight, height, moisture content, vigour of soybean. In addition, the tenacity of adherence of thiamethoxam by amphiphilic polymeric formulations on seed coat is reported.

*Corresponding author E-mail: jitendrakumar7@yahoo.com

MATERIALS AND METHODS

Seed coat agents: Amphiphilic nano-polymers were synthesized in our laboratory using different molecular weight PEG (1000, 2000, 4000) as hydrophilic head and aliphatic di-acids and aromatic di-esters namely azelaic acid, sebacic acid, dimethyl terephthalate and dimethyl isophthalate as hydrophobic moiety using conc. H_2SO_4 as catalyst[10]. The synthesized polymers Poly [poly (oxyethylene-1000)-oxy azelaoyl] (A1), Poly [poly (oxyethylene-2000)-oxy azelaoyl] (A2), Poly [poly (oxyethylene-4000)-oxy azelaoyl] (A-4), Poly [poly (oxyethylene-1000)-oxysebacoyl] (S1), Poly [poly (oxyethylene-2000)-oxysebacoyl] (S2), Poly [poly (oxyethylene-4000)-oxysebacoyl] (S4), Poly [poly (oxyethylene-1000)-oxyisophthaloyl] (I1), Poly [poly (oxyethylene-2000)-oxyisophthaloyl] (I2), Poly [poly (oxyethylene-4000)-oxyisophthaloyl] (I4), Poly [poly (oxyethylene-4000)-oxyterephthaloyl] (T1), Poly [poly (oxyethylene-2000)-oxyterephthaloyl] (T2), Poly [poly (oxyethylene-4000)-oxyterephthaloyl] (T4) were used to encapsulate thiamethoxam to prepare formulations namely A1F, A2F, A4F, S1F, S2F, S4F, I1F, I2F, I4F, T1F, T2F and T4F respectively. The micelle size of the formulated materials varied from 51.6 nm to 206.7 nm[10].

Solvents: HPLC grade acetonitrile, water and methanol and various other AR grade solvents were used**.**

Pesticide: Thiamethoxam technical grade, 96.5%, m/m and the commercial formulation, Cruiser® 35 FS was obtained through the courtesies of United phosphorous Ltd., India and Syngenta India Limited respectively.

Soybean seed: Soybean variety *Kuber* was obtained from *Pulse* Research *Laboratory, Division of Genetics,* Indian Agricultural Research Institute, New Delhi, India.

Seed coating: Required quantities of the different thiamethoxam nano-formulations (1% of the seed weight) were taken into a rotary evaporator flask and acetone was added to each. Soybean seeds (200 g) were introduced into the flask and tumbled till coated properly with the polymer composition. The coated seeds were immediately transferred to an aluminum foil, spread uniformly and separated manually to prevent clusters. The seeds were air-dried with the help of a warm air blower. Acetone was chosen as solvent due its ability to dissolve polymers and the formulations and its low toxicity towards soybean seeds.

Coating efficiency: To evaluate the thiamethoxam content on the soybean seed coats i.e. the coating efficiency, 10 g of coated seeds were immersed into 30 ml hexane-acetone (1:1 v/v). The containers were agitated in an ultrasonic bath for 10 min and the content filtered. The procedure was repeated thrice with fresh solvent mixture. The filtrates were combined and the solvent removed. The residue was dissolved in 15 ml acetonitrile. This solution was cleaned up by passing through Lichrolut filter RP-18 cartridge and analyzed for thiamethoxam by HPLC. The percent thiamethoxam recovery ranged from 86.1-93.2%.

Thiamethoxam retention study by the coated seed: To determine the tenacity with which different carriers held thiamethoxam, water release study was performed[11]. Ten g of treated seed was taken a 100 ml bottle containing 50 ml HPLC grade water. For the first sampling, each bottle containing seeds in water was inverted about 100 times. Then they were placed on a platform shaker and agitated. Aliquots of 1 ml each were withdrawn at 1, 3, 5, 7, 10, 14, 24 h and filtered through a 0.45 µ PTFE filter and the requisite volume (20 µl) was injected into an HPLC.

Chromatographic conditions: The HPLC conditions for estimation of thiamethoxam were standardized to avoid interference of polymers employing a solvent delivery module Varian ProStar model 240 as chromatographic pump. UV-visible detector was used at λ_{max} 254 nm. Isocratic elution was carried out in a RP-18 column with acetonitrile-water (70:30 v/v) mobile phase at a flow rate of 0.5 ml min^{-1}. An aliquot of 20 µl was injected in the HPLC. The retention time of thiamethoxam and polymers were recorded 4.81 and 3.9 min, respectively.

Analysis of release data

Determination of the diffusion exponents of the thiamethoxam in seed coats: The diffusion exponents from release data was calculated with the semi-empirical power law equation[12].

$$M_t / M_o = Kt^n \tag{1}$$

Where M_t/Mo is the fraction of active ingredient released at time t, K is a constant that incorporates characteristics (porosity, tortuosity) of the macro molecular network system and the active ingredients, and n, a diffusion parameter which is indicative of the transport mechanism. The model has been fitted by taking logarithm on both sides of Equation 1:

$$\log_e M_t/M_o = \log e\ K + n \log_e t + e \tag{2}$$

The values of K and n were determined from thiamethoxam release data.

In order to compare the release profile of different formulations with possible difference in release mechanisms (n values), a mean dissolution time[13] (MDT) was calculated using the following equation.

$$MDT = (n/n+1).\ K^{-1/n} \tag{3}$$

Where n= release exponent and K= release rate constant

Mean dissolution time (MDT) value is used to characterize thiamethoxam release rate from formulations and indicates the pesticide release retarding efficiency of polymers.

Calculation of $t_{1/2}$ (time taken for release of 50% of initial thiamethoxam from seed coats): The previously introduced exponential relation ($M_t / M_o = Kt^n$) may be used to calculated the time taken for release of 50% of initial thiamethoxam for test controlled release formulations and from commercial formulation.

At time taken for release of 50%, the equation (1) will be,

$0.5 = K(t_{1/2})^n$ (as at $t_{1/2}$ M_t=1/2 M_o)

$(t_{1/2})^n = 0.5/K$

$$t_{1/2} = (0.5/K)^{1/n} \quad (4)$$

Similar way time taken to release of 25%, 75% and 90% from the test formulation is calculated using M_t / M_o value as 0.25, 0.75 and 0.90 respectively.

The data were analyzed using SAS PROC REG

Seed quality assessment: After coating of the seed, the suitability of different polymers and formulations for seed coating was assessed based on the effect on different seed quality parameters *viz*., seed germination, seed vigour, seed moisture and seed storability.

Seed germination: The germinability of seeds was determined by using the between paper method[14]. Fifty seeds in three replications of were placed between two layers of moist germination papers with the help of counting board. The germination paper was soaked overnight in water. The germination papers were then rolled carefully without disturbing the position of seeds. They were then wrapped in a sheet of wax paper to reduce the surface evaporation of moisture from germination papers and placed in germinators at 27ºC temperature in an upright position. After five d a preliminary germination count was taken. At eight d of germination, germinated seeds were evaluated for normal seedlings, abnormal seedlings and dead seeds. The result of the germination test was calculated as the ratio of number of normal seedling to the total number of seeds and it was expressed as percentage.

Seedling length: At 8th d of germination, ten normal seedlings were selected randomly in each treatment from all the replications on eighth d. The shoot length was measured from the base of the primary leaf to the base of the hypocotyls and the mean shoot length was expressed in centimetre.

Ten normal seedlings used for shoot length measurement, were also used for the measurement of root length. The root length was measured from the tip of the primary root to base of hypocotyls and the mean root length was expressed in centimetre.

Fresh weight: Seedlings used for recording seedling length from each replication were subsequently weighed after removing the seeds and the fresh weight was expressed in mg per ten seedlings.

Dry weight: Seedlings used for recording seedling length from each replication were subsequently dried in oven in 85±1 °C for 24 h after removing the cotyledons and the dry weight was expressed in mg per ten seedlings[14].

Seed vigour: Vigour of the coated seeds was assessed based on germination percentage; seedling length and seedling dry weight. *Vigour index* 1(VI 1) was calculated using the following formula[15]:

Vigour index 1(VI 1)= Germination (%) × Seedling length

Thus obtained seedling dry weight and germination percentage was used to calculate the *Vigour Index* (VI 2) using the following formula:

Vigour Index 2 (VI 2)= Germination (%) × Seedling dry weight (mg)

Seed moisture content: Seed moisture content of the stored seeds was assessed at 3 months interval in three replicates by oven drying the coarsely ground seeds at 103 °C for 17 h as per ISTA (2004) method[14].

Seed storage studies: In seeds storage studies, the effect of different nano-formulated thiamethoxam on seed quality parameters and the efficacy of the polymers as carriers of pesticide was assessed in comparison to commercial and uncoated seeds. For storage studies, the coated and uncoated (control) seeds 300 g were kept in glass bottle and stored under ambient condition. Observations were taken on different seed quality assessment attributes during 6 months storage at 3 months time intervals as above.

Analysis of data: The percent seed germination data was transformed into Arcsine values and subjected to one way classified ANOVA by Tukey's HSD for multiple comparisons using SAS package. Other seed quality parameters were analysed in same manner without any transformation.

RESULTS AND DISCUSSION

Thiamethoxam retention by the coated seed: Loss of thiamethoxam in water from the soybean seed coats as a function of time is depicted in Figure 1 and Figure 2. The rate of loss of thiamethoxam from the coated seeds with controlled release formulations was much slower than the commercial formulation (Cruiser® 35 FS). During the first h of retention study, maximum loss of 62.1% was noted obtained from the commercial formulation. In case of controlled release formulations, the maximum loss was observed in I4F to the tune of 48.2% and maximum quantity of thiamethoxam was retained by A2F to the tune of 67.3% after first h of release study. At the completion of study (24 h) the maximum loss was less than 60% for all the developed formulations where as the commercial formulation released more than 70% of a.i. from the soybean seed coat. Thiamethoxam retention after 24 h of study followed the following order:

CF< I1F< I2F<I4F<A1F<T1F<T2F=T4F<A4F= S2F<S1F< A2F<S4F

Release trends from seed coats were found to be similar for all controlled release formulations with little variation. The release of thiamethoxam from seed coats showed steep increase in release rate till 3rd h of study. There after, it increased in a decreasing order till end of the study. The release rates after the 3rd h of study were constant or with a little variation for all the controlled release formulations. This may be due to much adherence capacity of polymers to seed. Another reason may be the slow diffusion of thiamethoxam from controlled release formulations.

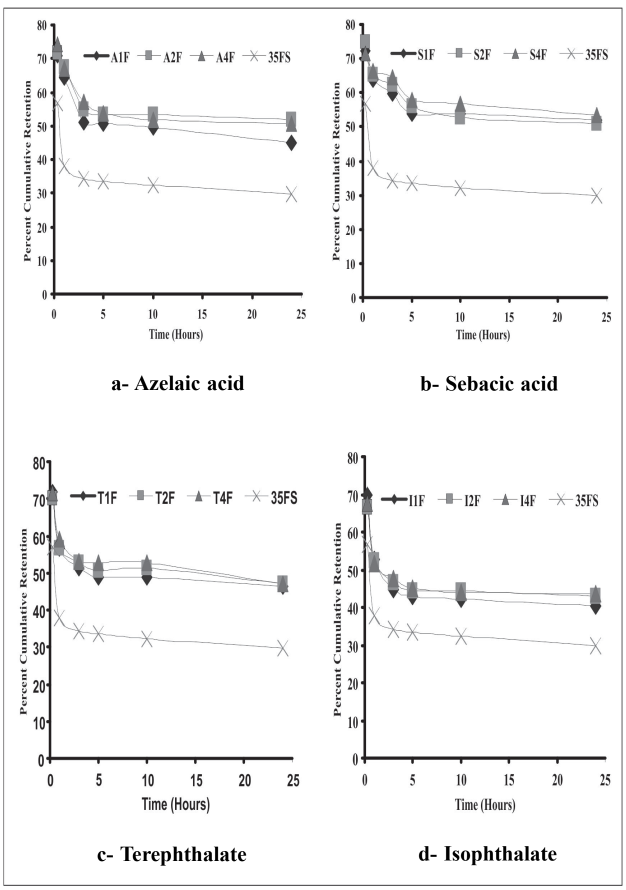

Figure 1. Percent cumulative retention of thiamethoaxm in seed coats with different formulations having same linker molecules 1a- azelaic acid, 1b- sebacic acid, 1c- terephthalate, 1d- isophthalate

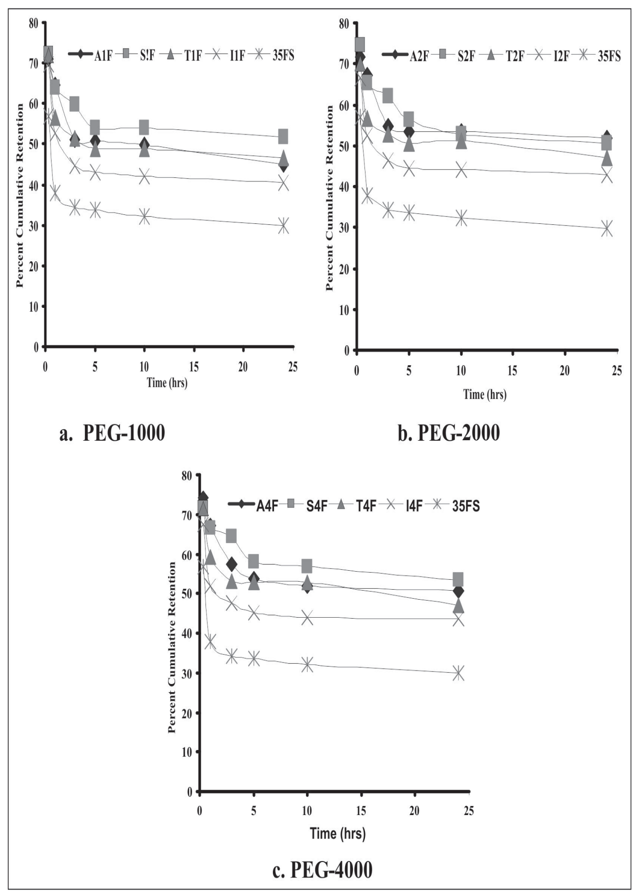

Figure 2. Percent cumulative retention of thiamethoaxm in seed coats with respect to polymers having same PEG moiety 2a-PEG-1000, 2b-PEG-2000, 2c-PEG-4000

Diffusion exponents of loss of thiamethoxam from seed coats in water: To simplify the analysis from three-dimensional devices, the data of loss of thiamethoxam from seed coat were analyzed by applying the empirical equation proposed by Ritger and Peppas, 1987[12]. The parameters (K and *n*) obtained from thiamethoxam loss from seed coat in water are presented in Table 1.

Table 1. The constants derived from fitting data during retention of thiamethoxam in seed coated with controlled release and commercial formulations in the empirical equation $M_t/M_o = Kt^n$.

Formulation	Korsmeyer-peppas		
	R^2	K	*n*
A1F	0.954	0.368	0.202
A2F	0.957	0.348	0.194
A4F	0.996	0.333	0.210
S1F	0.972	0.348	0.166
S2F	0.952	0.325	0.176
S4F	0.917	0.329	0.129
T1F	0.902	0.391	0.187
T2F	0.886	0.397	0.156
T4F	0.908	0.379	0.164
I1F	0.914	0.430	0.202
I2F	0.916	0.440	0.165
I4F	0.881	0.435	0.163
35 FS	0.812	0.559	0.134

According to correlation coefficients, it is deduced that the loss profiles of thiamethoxam from seed coats fit well to the empirical equation. The *n* values ranged from 0.129 to 0.210 for the controlled release formulations. For the commercial formulation, it was 0.134**.** K values obtained from the equation ranged from 0.325 to 0.440 for the controlled release formulation seed coats. For the case of spherical sample, $0.43 \leq n$ corresponds to a Fickian diffusion mechanism, $0.43 < n < 0.85$ to non-Fickian transport, $n=0.85$ to Case II (relaxational) transport, and $n > 0.85$ to super case II transport[12]. So it can be concluded that loss of thiamethoxam from the controlled release formulations and commercial formulation follow Fickian or quasi Fickian diffusion kinetics. The difference in *n* values may be due to the different chemical nature of the polymers. With the change in hydrophilic and hydrophobic segment of polymers, small change in *n* values was obtained. K values obtained from the equation ranged from 0.325 to 0.440 for controlled release formulation seed coats.

The half lives (i.e. time taken for 50% loss) of thiamethoxam loss from seed coats in water for both developed controlled release formulations and commercial formulation are reported in Table 2. The $t_{1/2}$ of thiamethoxam in various test polymers ranged from 2.11 to 25.84 h. The $t_{1/2}$ in commercial formulation was only 0.44 h. In order of increasing $t_{1/2}$ of thiamthoxam in various formulations, the following order was observed:

35FS < I1F < I2F < I4F <TIF < T2F <A1F < T4F < A2F <A4F < S1F < S2F < S4F

The time for 50% release of thiamethoxam from seed coats for the commercial formulation is lower than the controlled release formulations. This is mainly because the a.i. in controlled released formulations is encapsulated in polymeric nano spheres where as the commercial product is in the form of FS. In case of commercial formulation, thiamethoxam release was instantaneous. It was observed that with increase in hydrophilic chain length of amphiphilic polymers having same di-acids, $t_{1/2}$ value of loss of thiamethoxam from seed coat increased. This indicates that the amphiphilic polymers with higher hydrophilic segment retain thiamethoxam more efficiently.

The Mean dissolution time (MDT) of loss of thiamethoxam from seed coats in water for both the developed and the commercial formulations is reported in Table 2. In all cases the MDTs of the developed formulations were higher than the commercial formulation. The formulation namely S4F showed the best release retarding

Table 2. Dissolution parameter of test formulations

Formulation	$t_{0.25}$	$t_{0.50}$	$t_{0.75}$	$t_{0.90}$	MDT
A1F	0.15	4.57	34.06	84.07	23.80
A2F	0.18	6.48	52.36	134.01	37.48
A4F	0.26	6.92	47.57	113.21	32.46
S1F	0.14	8.90	101.82	304.58	81.84
S2F	0.22	11.44	113.91	320.21	87.25
S4F	0.12	25.84	600.28	2469.65	638.58
T1F	0.09	3.72	32.57	86.33	23.89
T2F	0.05	4.41	59.30	190.83	50.60
T4F	0.08	5.44	64.05	194.17	52.04
I1F	0.07	2.11	15.63	38.44	10.89
I2F	0.03	2.18	25.47	76.99	20.65
I4F	0.03	2.34	28.02	85.58	22.90
35 FS	0.00	0.44	9.04	35.33	9.16

efficiency. In order of increasing MDT value the developed formulation can be arranged as follows:

35FS < I1F < I2F < I4F <A1F < T1F < A4F < 2F <T2F < T4F < S1F < S2F < S4F

Mean dissolution time (MDT) was used to characterize thiamethoxam release rate and indicates the thiamethoxam release retarding efficiency of the polymers. Higher the MDT value means that their will be higher retardation by the polymer to release the active ingredient. Better controlled release formulation will be one which has higher MDT value. It was seen that as the chain length of hydrophilic segment increased except A4F, the values of MDT also increased implying better thiamethoxam release retarding efficiency.

The formulation namely S4F showed the best loss retarding efficiency. In general polymers with aliphatic hydrophobic segment namely A1F, A2F, A4F, S1F, S2F and S4F showed better MDT values than polymers with aromatic hydrophobic segments namely T1F, T2F, T4F, I1F, I2F and I4F. All the developed formulations prepared from both aliphatic and aromatic polymers showed better release retarding efficiency than the commercial formulation. Thus it can be concluded that the developed formulations will reduce the loss of pesticide from seed coat and will increase its bioavailability. At the same time seed treatments with developed controlled release formulations will overcome the problems of environmental contamination.

Seed quality enhancement: Irrespective of the treatment, the seed quality parameters declined progressively with the increase in storage period. Different seed quality parameters, *viz.* seed germination, seed vigour, seed moisture and seed storability were better for the coated seeds with controlled release formulations rather than control seeds and seeds treated with commercial formulation. The higher seed quality parameters observed with the neonicotinoid treated seeds may be due to its phytotonic effect[16]. Better storability of imidacloprid treated sugarbeet seeds was also reported[17].

Germination: Effect of thiamethoxam based controlled release formulation coats on germination of soybean seed is reported in Table 3. The percentage germination reduced over a period of six months in both coated and uncoated seeds. Germination retaining capability for CR formulation coated seeds was always better than the uncoated and commercial formulation coated seeds. Statistical analysis revealed non-significant differences among the treatments including control and commercial formulations in germination count at zero months and after 3 months. At six months of storage, treatments were significantly different from each other with respect to germination percentages. After six months percent germination reduced drastically from 97.32 to 73.69% in case of uncoated seeds, 97.74 to 80.78% for commercial formulation treated seeds. Whereas, developed nano-formulations coated seeds showed germination in the range of 92.97-77.12, 44% after 6 months of storage. Their transformed values also followed the same trend. After 3 months of storage, A4F coated seeds performed best with 99.6% of germination. The same formulation coated seeds performed best with the germination of 92.97% after 6 months of storage.

In order of increasing germination percentage at six month the developed formulations followed the order:

Control<T1F<A1F<I1F<35FS<S1F<S2F<T2F <A2F<T4F <S4F<I2F<I4F<A4F

The germination of soybean seeds declined progressively with increase in the period of storage in all the treatment combinations, which may be attributed to the phenomenon of ageing and depletion of food reserves and decline in synthetic activity of seed apart from death of seed because of fungal invasion, insect damage, fluctuating temperature, relative humidity and storage container in which seeds are stored. It may be due to appreciable decrease in peroxidase activity with ageing which makes the seed more sensitive to the effects of oxygen and free radicals in membrane, unsaturated fatty acids and the production of secondary lipid peroxidation products such as monoaldehyde and lipid conjuents. It was reported that the loss of viability with enzyme activity are associated in barley[18] and in sorghum[19]. It was seen that seeds coated with controlled release formulations which contained amphiphilic polymers showed better germination percentage at six months of study than commercial formulation and control. Higher germination percentage of coated seeds may be attributed to polymeric nature of coat. It was reported that seed coating with polymers is highly beneficial for enhancing germination as seed coats become impermeable to oxygen[6,20]. Coating with a hydrophilic polymer regulated the rate of water uptake, reduced imbibitional damage and improved the emergence of soybean seeds[7]. The results of the study are in conformity with the findings in maize[21] and groundnut[22].

Table 3. Effect of thiamethoxam based seed coats on germination, fresh and dry weights of soybean seed

	Germination			Fresh weight			Dry weight		
Treatment	0 month	3 month	6 month	0 month	3 month	6 month	0 month	3 month	6 month
A1F	98.33 (5.69)	91.94(5.50)	79.65^{bc}(5.12^{bc})	8.97^{abcde}	7.79^{cd}	7.32^{c}	0.42^{abc}	0.32^{cd}	0.30^{bc}
A2F	99.5(5.72)	95.36(5.60)	84.08^{ab}(5.26^{ab})	8.02^{e}	7.56^{d}	7.22^{c}	0.41^{bc}	0.35^{bcd}	0.33^{bc}
A4F	100(5.74)	99.60(5.73)	92.97^{a}(5.53^{a})	9.47^{abc}	9.34^{ab}	8.67^{ab}	0.53^{a}	0.51^{a}	0.51^{a}
I1FF	97.65(5.67)	96.60(5.64)	80.24^{bc}(5.14^{bc})	8.32^{cde}	7.69^{cd}	7.18^{c}	0.43^{abc}	0.33^{bc}	0.31^{bc}
I2F	99.35(5.72)	92.50(5.51)	86.11^{ab}(5.32^{ab})	8.57^{cde}	8.11^{bcd}	7.77^{c}	0.51^{ab}	0.45^{ab}	0.42^{ab}
I4F	99.41(5.72)	96.20(5.63)	86.38^{ab}(5.33^{ab})	10.12^{a}	9.66^{a}	9.32^{a}	0.52^{ab}	0.32^{cd}	0.30^{bc}
S1F	99.35(5.72)	98.20(5.68)	81.10^{bc}(5.16^{bc})	8.75^{bcde}	7.29^{d}	7.21^{c}	0.43^{abc}	0.37^{bcd}	0.35^{bc}
S2F	99.42(5.72)	92.00(5.50)	81.64^{bc}(5.18^{bc})	8.23^{de}	7.77^{cd}	7.43^{c}	0.49^{abc}	0.39^{abcd}	0.33^{bc}
S4F	99.96(5.74)	91.88(5.50)	86.02^{ab}(5.32^{ab})	8.62^{cde}	8.16^{bcd}	7.82^{bc}	0.46^{abc}	0.40^{abcd}	0.38^{bc}
T1F	98.5(5.69)	88.53(5.39)	77.12^{bc}(5.03^{bc})	7.90^{e}	7.43^{d}	7.19^{c}	0.43^{abc}	0.37^{bcd}	0.35^{bc}
T2F	99.52(5.72)	93.54(5.55)	82.94^{bc}(5.22^{bc})	8.14^{de}	7.68^{cd}	7.34^{c}	0.50^{abc}	0.44^{abc}	0.41^{ab}
T4F	99.29(5.72)	97.57(5.67)	85.57^{ab}(5.31^{ab})	9.26^{abcd}	8.80^{abc}	8.79^{a}	0.50^{abc}	0.44^{abc}	0.41^{ab}
Commercial	97.74(5.67)	89.61(5.43)	80.78^{bc}(5.15^{bc})	8.29^{cde}	7.49^{d}	7.27^{c}	0.43^{abc}	0.34^{bcd}	0.33^{bc}
Control	97.32(5.66)	92.39(5.51)	73.69^{c}(4.92^{c})	9.84^{ab}	7.51^{d}	7.04^{c}	0.39^{C}	0.29^{d}	0.27^{c}
CV	1.15(0.58)	5.56(2.83)	3. 84(1.95)	4.55	5.13	3.72	8.59	10.19	12.21
PROB > F	0.102(0.104)	0.33(0.337)	<.0001(<.0001)	<.0001	<.0001	<.0001	0.0013	<.0001	.0001
TUKEY'S HSD	NS(NS)	NS(NS)	9.5593(0.3053)	1.199	1.2379	0.8599	0.1192	0.1173	0.1308

Means with same letter are not significantly different. Values in parentheses are Arcsine transformed values

Fresh and dry weights of seedlings: Change in fresh and dry weights was observed with storage life of seeds for both coated and uncoated seeds (Table 3**)**. Statistical analysis revealed significant differences in fresh and dry weights for both coated and uncoated seeds during 6 months of storage. After 6 months, the highest fresh and dry weights were observed in treatments I4F and A4F, respectively. In order of increasing fresh weight at six months the developed formulations can be arranged as:

Control<I1F<T1F<S1F<A2F<35FS<A1F<T2F<S2F<I2F<S4F <A4F<T4F<I4F

In order of increasing dry weight at six months the developed formulation can be arranged as:

Control<I4F=A1F<I1F<A2F=S2F=35FS<T1F=S1F<S4F<T2F =T4F<I2F<A4F

Fresh and dry weights of seedling decreased with increase in storage period. This may be due to ageing, which resulted in deterioration of seed. Lower extent of decrease in case of coated seeds may attributed to the role of seed coats which maintained favorable environment inside the seeds by maintaining relative humidity of storage environment, reducing the ageing of seeds and preventing the diffusion of gases between seed and outside environment[20]. Higher fresh and dry weight of coated seeds can also be attributed to presence of thiamethoxam in formulations. It has been reported that the weight of soybean seedlings increased after 15 d by using thiamethoxam as seed treatment[23]. Higher fresh and dry weights of seedlings from seeds coated with controlled release formulations than commercial formulation may attribute to combined effect of thiamethoxam and polymer coating.

Shoot and root lengths: During storage of soybean seeds, changes in shoot and root lengths were recorded and are presented in Table 4. Treatments with regard to shoot length were significantly different from each other during 3 and 6 months of storage. Root length as well as total seedling length had responded significantly to the treatments during the entire period of experimentation. Longest seedling length (40.73 cm) was observed for I4F after 6 months of storage. At that period, control had only 28.24 cm seedling length. In order of increasing shoot length at six months the developed formulation followed the order:

Control<35FS<T2F<T4F<A4F<S1F<I1F<I2F<A2F<S2F <S4F<A1F<T1F<I4F

In order of increasing root length at six months the developed formulation can be arranged as:

Control<35FS<T2F<A4F<S1F<A2F<I1F<T4F<S2F<I2F <S4F<A1F<T1F<I4F

The root and shoot lengths of soybean seedlings decreased gradually with advancement in storage period. This may be attributed to age induced decline in germination. The damage caused by fungi and insects and also toxic metabolites which may have hindered the seedling growth, and similar findings have been also reported in mungbean[24]. It was observed that the controlled release formulations showed higher seedling length than control and the commercial formulation. It may be due to polymer film act as a physical barrier thus reducing the loss of nutrients from the seed coverings and restricting the oxygen diffusion to the embryo, and there by restricting the ageing effect.

Seed vigour: Seed vigour indices are presented in Table 5. Statistical analysis revealed non-significant differences among the treatments including control and the commercial formulation in Vigour Index-1 at zero months and after 3 months. But seed coated with controlled release formulations showed better seed vigour as compared to control and the commercial formulation coated seeds after six months of storage. Vigour Index-1 was found to be lowest for control seeds followed by commercial formulation coated seeds. Vigour index-1 was reduced from 4541.19 to 2080.48 in control seeds and from 4587.08 to 2693.56 in commercial formulation coated seeds at the end of 6 months storage. In case of controlled release formulations coated seeds, Vigour Index-1 ranged from 3519.06 to 2942.99 after 6 month of storage. Seeds coated with I4F formulation had the highest vigour after 6 months of storage. In order of increasing Vigour Index-1 at six months the developed formulation can be arrange as follows:

Control<35FS<T2F<I1F<T1F<S1F<A1F<S2F<A2F< T4F<I2F<S4F<A4F<I4F

The vigour indices of soybean seeds decreased gradually with advancement in storage period. The effect of controlled release formulations as seed coat on Vigour Index-1 was apparent after 3 months of study, where as effect on Vigour Index-2 was observable during the entire period of study. Significantly higher Vigour Index-1 and Vigour Index-2 were recorded in seeds coated with controlled release formulations at six months of study. Increase in Vigure Index-1 may be attributed to higher germination percentage and seedling length recorded in case controlled release formulations treated seeds, where as increase in Vigour Index-2 may be attributed to higher germination percentage and seedling dry weight maintained by controlled release formulations treated seed. Seed Vigour Index-2 responded to treatments significantly during entire period of experimentation. Highest Vigour Index-2 was obtained in A4F at both the initial time as well as after 6 months of storage. In order of increasing Vigour Index-2 at six months the developed formulations can be arranged as follows:

Control<A1F<I1F<I4F<T1F<S2F<35FS<A2F<S1F< S4F<T2F<T4F<I2F<A4F

Moisture content: Moisture content in seeds over the six months of storage increased in both coated and uncoated seeds and is presented in the Table 5. It varied between 9.75 to 9.06% during the initial period of experiment for all the treatments. After 6 months of storage, moisture content was increased and found to be highest in case of control. Whereas, coated seeds maintained moisture contents in seed at preferable level. In order of decreasing moisture at six month the developed formulation can be order as:

Control>35FS>A1F>S1F>S2F>I2F>A2F=A4F=S4F= T1F>I1F.I4F=T4F>T2F

The moisture content of seeds increased as the storage period advanced up to six months. This may be due to hygroscopic nature of soybean seeds. It gained moisture from surrounding environment. Higher moisture content of seed deteriorates the seed quality. As moisture enhances, various enzymatic processes which lead to degradation of protein and other food reserve resulting in reduction of vigour and germination. It was seen that controlled release formulations treated seeds showed lower moisture content. This is because of amphiphilic nature of the polymers present in the test formulations which may act as physical barrier to moisture [25].

Effective polymeric carriers have thus been identified for thiamethoxam based seed coats to improve the storage of soybean seed. The coated seeds with different nano-formulations had better seed quality over control and the commercial formulation coated seeds. Thiamethoxam retention on soybean seed coats was more with nano-encapsulated formulations than the commercial formulation. Higher retention of thiamethoxam will reduce loss of pesticide and will increase its bioavailability. Seed treatments with controlled release formulations will mitigate the problem of environmental contamination.

Table 4. Effect of thiamethoxam based seed coats on shoot and root lengths of soybean seed

	Shoot length			Root length		
Treatment	0 month	3 month	6 month	0 month	3 month	6 month
A1F	22.41	20.12^{a}	19.18^{a}	20.88	20.13^{a}	18.92^{ab}
A2F	22.04	20.68^{a}	18.78^{a}	21.41	20.09^{a}	18.26^{ab}
A4F	23.23	18.79^{a}	18.53^{a}	22.58	20.79^{a}	17.91^{ab}
I1F	21.21	19.56^{a}	18.70^{a}	21.27	20.28^{a}	18.29^{ab}
I2F	23.33	19.43^{a}	18.76^{a}	22.56	19.61^{a}	18.57^{ab}
I4F	23.53	21.13^{a}	19.83^{a}	21.38	20.37^{a}	20.90^{a}
S1F	23.1	19.45^{a}	18.69^{a}	23.71	19.43^{ab}	18.17^{ab}
S2F	22.27	20.91^{a}	18.95^{a}	21.87	20.49^{a}	18.55^{ab}
S4F	22.52	19.06^{a}	19.03^{a}	22.53	19.37^{ab}	18.89^{ab}
T1F	23.03	19.40^{a}	19.50^{a}	24.54	19.74^{a}	19.26^{ab}
T2F	21.7	18.55^{a}	17.84^{a}	22.08	19.95^{a}	17.62^{ab}
T4F	24.47	18.36^{ab}	18.45^{a}	24.21	20.07^{a}	18.35^{ab}
Commercial	24.69	18.42^{ab}	15.99^{ab}	22.28	18.20^{ab}	17.26^{ab}
Control	23.02	15.02^{b}	12.56^{a}	23.63	16.64^{b}	15.68^{b}
CV	12.27	5.95	8.63	9.31	4.88	6.99
PROB > F	0.9722	0.0002	0.0009	0.5681	0.002	0.0274
TUKEY'S HSD	NS	3.4397	4.7278	NS	2.8832	3.856

Means with same letter are not significantly different.

Table 5. Effect of thiamethoxam based seed coats on Vigour index-1, Vigour index-2 and moisture content of soybean seed

	Vigour Index-1			Vigour Index-2			Moisture content (%)		
Treatment	0 month	3 month	6 month	0 month	3 month	6 month	0 month	3 month	6 month
A1F	4253.13	3699.12^{ab}	3040.87^{ab}	41.05^{bc}	29.99^{cd}	23.82^{cd}	9.75 (1.79)	9.85^{a} (1.80^{a})	$10.49^{a}(1.85^{a})$
A2F	4323	3889.71^{a}	3119.74^{ab}	41.25^{bc}	33.99^{bcd}	27.57^{bcd}	9.36(1.75)	9.65^{ab} (1.78^{ab})	$10.09^{ab}(1.82^{ab})$
A4F	4580.67	3942.18^{a}	3387.55^{ab}	53.42^{a}	50.62^{a}	47.59^{a}	9.30(1.75)	9.65^{ab} (1.78^{ab})	$0.09^{ab}(1.82^{ab})$
I1F	4147.79	3847.70^{a}	2978.59^{ab}	41.78^{abc}	32.48^{bcd}	24.78^{bcd}	9.35(1.75)	9.63^{ab} (1.78^{ab})	$0.07^{ab}(1.82^{ab})$
I2F	4556.74	3609.16^{ab}	3212.26^{ab}	50.44^{ab}	41.41^{abc}	36.27^{ab}	9.48(1.76)	9.76^{a} (1.79^{a})	$10.20^{a}(1.83^{a})$
I4F	4465.82	3989.35^{a}	3519.06^{a}	51.44^{ab}	31.09^{bcd}	25.75^{bcd}	9.33 (1.75)	9.61^{ab} (1.78^{ab})	$0.05^{ab}(1.82^{ab})$
S1F	4646.81	3816.54^{a}	2992.62^{ab}	43.09^{abc}	36.87^{bcd}	28.16^{bcd}	9.45(1.76)	9.96^{a} (1.81^{a})	$10.40^{a}(1.85^{a})$
S2F	4387.89	3813.97^{a}	3073.13^{ab}	48.34^{abc}	35.61^{bcd}	27.06^{bcd}	9.22(1.74)	10.05^{a} (1.82^{a})	$10.23^{a}(1.83^{a})$
S4F	4502.79	3531.08^{ab}	3263.63^{ab}	46.40^{abc}	37.06^{bcd}	32.37^{bc}	9.41(1.76)	9.65^{ab} (1.78^{ab})	$0.09^{ab}(1.82^{ab})$
T1F	4689.37	3474.35^{ab}	2988.97^{ab}	42.84^{abc}	32.74^{bcd}	26.78^{bcd}	9.32(1.75)	9.65^{ab} (1.78^{ab})	$0.09^{ab}(1.82^{ab})$
T2F	4354.61	3597.64^{ab}	2942.99^{ab}	49.51^{abc}	41.38^{abc}	34.11^{bc}	9.06(1.72)	8.76^{b} (1.69^{b})	9.20^{b} (1.74^{b})
T4F	4823.08	3750.98^{a}	3149.57^{ab}	49.66^{abc}	43.08^{ab}	35.41^{bc}	9.33(1.75)	9.61^{ab} (1.78^{ab})	$0.05^{ab}(1.82^{ab})$
Commercial	4587.08	3287.41^{ab}	2693.56^{bc}	42.48^{abc}	30.45^{bcd}	27.10^{bcd}	9.43(1.76)	9.71^{a} (1.78^{a})	$10.75^{a}(1.88^{a})$
Control	4541.19	2922.20^{b}	2080.48^{c}	37.67^{c}	27.13^{d}	19.70^{d}	9.35(1.75)	9.99^{a} (1.81^{a})	10.95^{a} (1.90^{a})
CV	9.57	7.11	8.79	8.75	11.7	13.3	2.79(1.41)	3.2(1.58)	3.07(1.51)
PROB > F	0.8791	0.0023	0.0003	0.0007	<.0001	<.0001	0.46(0.47)	0.009(0.006)	0.0003(0.0002)
TUKEY'S HSD	NS	781.83	801.66	12.029	12.669	11.9	NS(NS)	0.931(0.084)	0.943(0.083)

Means with at least one letter common are not statistically significant using Minimum Significant Difference. Values in parentheses are Arcsine transformed values.

REFERENCES

1. Silininger PJ, Van Cauwenberge JE, Bothast RJ, Weller DM, Thomashaw LS and Cook RJ (1996) Effect of growth culture physiological state, metabolites, and formulation on the viability, phytotoxicity, and efficacy of the take-all biocontrol agent *Pseudomonas fluorescens* 2-79 stored encapsulated on wheat seeds. *Appl Microbiol Biotechnol* **45**: 391-398.
2. Cliquet S and Scheffer RJ (1997) Influence of culture conditions on growth and survival of conidia of *Trichoderma* spp. coated on seeds. *Biocontrol Sci Technol* **7**: 171-181.
3. Shah-Smith DA and Burns RG (1997) Shelf-life of a biocontrol *Pseudomonas putida* applied to sugar beet seeds using commercial coatings. *Biocontrol Sci Technol* **7**: 65-74.
4. Jeffs KA and Tuppen RJ (1986) Applications of pesticides to seeds: Requirements for efficient treatment of seeds. In: *Seed Treatment*, KA Jeffs (Ed), British Crop Protection Council, Thornton Heath/Surrey, England, **3**: 17-45.
5. Kaufman G (1991) Seed coating: A tool for stand establishment, a stimulus to seed quality. *Hort Technol* **1**: 98-102.
6. Duan X and Burris JS (1997) Film coating impairs leaching of germination inhibitors in sugarbeet seeds. *Crop Sci* **37**: 515-520.
7. Hwang W D and Sung FJM (1991) Prevention of soaking injury in edible soybean seeds by ethyl cellulose coating. *Seed Sci Technol* 19: 269-278.
8. Johnson GA, Hicks DH, Stewart RF and Duan XM (1999) Use of temperature-responsive polymer seed coating to control seed germination. In: *Proc Sixth Symp on Stand Establishment and ISHS Seed Symp*, *Roanoke, Virgina, USA*, **504**: 229-236.
9. Lee-SheongChun, Chung-DhunHwa, Kim-JinHee, Song-Dong S, Lee S, Chung CH, Kim JH and Song DS (1996) Effects of polymer coating on seed vigour in rice. *Korean J Crop Sci* **41**: 274-285.
10. Sarkar DJ, Kumar J, Shakil NA, Adak T, and Watterson AC (2012) Synthesis and characterization of amphiphilic PEG based aliphatic and aromatic polymers and their self assembling behaviour. *J Macromol Sci A* **49**: 455-465.
11. Nisar K, Kumar J, Walia S, Shakil NA, A Kumar MB, Parsad R. and Parmar BS (2009). Development of polymeric seed coats based on azadirachtin-A. *Pest Manag Sci* **65**: 175-182.
12. Ritger PL and Peppas NA (1987) A simple equation for description of solute release I. Fickian and anomalous release from non swellable devices in the form of slabs, spheres, cylinders or discs. *J Control Rel* **5**: 23-36.
13. Mockel JE and Lippold BC (1993) Zero order drug release from hydrocolloid matrices. *Pharm Res* **90**: 1066-1070.
14. Anonymous (1996) International rules for seed testing. *Seed Sci Technol* **24** (Supplementary): 1-228.
15. Abdul-baki AA and Anderson JD (1973) Vigour determination in soybean by multiple criteria. *Crop Sci* **13**: 630-633.
16. Jarande NT and Dethe MD (1994) Effective control of brinjal sucking pests by imidacloprid. *Plant Protec Bull* **46**: 43-44.
17. Schaufele WR, Henneke K, Peleiderer UF, Lehenerj T, Schiffner F and Abel G (1996) Germination capacity and field emergence of imidacloprid treated sugarbeet seed after storage for one year. *Gesunde-Ptlanzen*. **48**: 206-209.
18. Harrison JG and Perry DA (1976) Studies on the mechanism of barley seed deterioration *Ann Appl Biol* **84**: 57-62.
19. Perl M, Luria I and Gelmond H (1978) Biochemical changes in sorghum seeds affected by accelerated ageing. *J Exp Bot* **29**: 497-509.
20. Sachs M, Cantliffe DJ and Nell TA (1981) Germination of clay coated sweet pepper seeds. *J Am Soc Hortic Sci* 106: 385-389.
21. Ni BR and Biddle AL (2001) Alleviation of seed imbibitional chilling injury using polymer film coating. In: *Seed Treatment Challenges and Opportunities, Proc Int Symp Wishaw,* New Warwickshire, UK*,* **13**: 73-80.
22. Chikkanna C S, Timmegouda and Ramesh R (2000) Hydrophilic polymer seed treatment on seed quality and yield in fingermillet, cowpea and groundnut. In: *Seeds and Farms*, pp 39-45.
23. Horii A, McCue P and Shetty K (2007) Enhancement of seed vigour following insecticide and phenolic elicitor treatment. *Bioresource Technol* **98**: 623-632.
24. Paul SR, Sharma NN, Sarma D, Borah RK and Nath PD (1996) Maintenance of viability and vigour of stored mungbean seeds under the ambient conditions in the hills zone of Assam. *Ann Agril Res* **17**:196-198.
25. McGee DC, Iles A and Misra MK (1989) Suppression of storage fungi in grain with soybean oil. *Phytopathol* **79**: 1140.

Manuscript No. PRJ/01/12-08
Received 17 January, 2012; Accepted 04 June, 2012