SYNERGISTIC EFFECT AND STABILITY OF A SEED TREATMENT FORMULATION COMPRISING STROBILURIN AND ESSENTIAL OILS ON GROUNDNUT (ARACHIS HYPOGAEA)

Kawar Lal Dabodhia[1], Narendra Pal Lamba[2], Manmohan Singh Chauhan[3*], Saurabh Pandey[4], Brijesh Tripathi[5], Seema Manchanda[6*], Rohit Bhatia[7]

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Abstract

Phytopathogenic fungi infesting plants and plant parts can cause significant damage to crops, resulting in huge losses in production. To prevent such damage, there is a need to develop effective fungicides that have curative, preventative, and systemic action to protect cultivated plants and their seeds. However, conventional fungicide formulations have drawbacks such as environmental pollution, high cost, and unsatisfactory performance. To overcome these challenges, combination of fungicides and essential oils are increasingly being formulated as seed treatment formulations. In this study we report the physical stability and efficacy of a seed treatment fungicide formulation containing Trifloxystrobin, Cashew nut oil, and Karanj oil under different storage conditions over a 24-month period. The formulations were studied under heat stability, cold stability, and room temperature storage conditions, and various parameters were analyzed, including active content, suspensibility, pH, particle size, pourability, specific gravity, and viscosity. The seed treatment formulation of Trifloxystrobin 3.5% + Cashew nut oil 15.0 + Karanj oil 22.5 FS (trial 6) found to be stable over the 24-month study period, while also showcasing its high efficacy against soil-borne fungi in Groundnut. The formulation's stability over time makes it a reliable and long-lasting solution for crop protection. Overall, the study highlights the effectiveness and stability of this seed treatment formulation, providing valuable insights for the development of safe and efficient crop protection solutions.

Keywords: Fungicide, Phytochemicals or Essential oil, Synergism, Strobilurin, Seed treatment (FS), Bio-efficacy, Stability, Groundnut, yield

[1,2,3*]Department of Chemistry, Amity University Rajasthan, Jaipur B. K. Birla College, Kalyan Maharashtra, India.
[4,5,6*]Directorate of Plant Protection, Quarantine & Storage (DPPQS), Ministry of Agriculture & Farmers Welfare, Haryana, India.
[7]Shrinathji Consultants, Delhi
Email: [1]kanwar409@yahoo.co.in

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1. Introduction

Agriculture is crucial for meeting the food demands of the growing global population, but protecting crops from pests without harming the environment is a major challenge faced by farmers worldwide [1]. Traditional chemical control methods have limitations, such as high cost and pollution, and developing resistant varieties has been slow and unreliable [1,27]. To address these challenges, there is an increased interest in developing management practices such as seed treatment [2-3], which can reduce inoculum potential, ensure sustainability, and maintain a healthy ecosystem. Seed is a vital input for sustainable agriculture, and seed treatment can effectively manage seedborne and early season diseases and pests, which can have devastating consequences if left unmanaged [4].

Seed treatment has become increasingly popular due to its ability to enhance crop yields by protecting seeds from pests and diseases both before and after planting. This cost-effective technique is applied directly onto the seed using advanced technology and offers a consistent stand across different soil types, cultural practices, and environmental conditions. Flowable seed treatment concentrates, similar to suspension concentrates, are commonly used and applied directly onto the seed using a fluid suspension product. The formulation includes gelling and thickening agents to optimize viscosity and ensure easy application, even at low temperatures. The benefits of using flowable seed treatments include water-based and water-dilutable properties, better seed retention, and ease of cleaning seed treatment equipment [5].

With the successful control of fungal diseases through seed treatment, farmers are now able to focus their efforts on breeding crops with other desirable attributes such as improved seed quality [6,23]. Seed treatment has become an important tool for protecting seeds and seedlings from diseases and pests that can damage crop emergence and growth. Commercial seed treatment using pesticides has been widely used for various crops, and the use of chemical treatments is likely to continue for the foreseeable future.

While conventional chemical fungicide formulations have been widely used in the past, they come with several significant drawbacks. One of the primary concerns is their potential for causing environmental pollution. Moreover, their high cost can make them inaccessible to many farmers, particularly those with limited resources. Additionally, unsatisfactory performance has been reported in some cases, indicating that the effectiveness of these formulations may be limited in certain situations. As a result, there is a growing need to develop alternative methods for protecting crops from fungal infestations that are both effective and environmentally friendly.

Essential oils have emerged as promising alternatives to chemical fungicides in recent years [7,22]. Produced during a plant's secondary metabolism, these bioactive compounds have good pesticidal potency [8,9]. However, the rapid degradation of botanical extracts caused by volatilization and photo degradation of the active principles, along with their low solubility towards target surfaces, limits their use. Essential oils are widely used in the medicinal, pharmaceutical, cosmetics, pesticide, and insecticide industries due to their role in a plant's defensive mechanisms against fungi, pathogenic microorganisms, herbivores, and insects. Jatropha, Garlic acid, Ginger, D-limonene, Citronella or Ceylon ironwood, Mahua, Pongamia/Karanja/Karanj, Cashew Nut Shell Liquid, and Turmeric are extensively used for controlling pests [10-14]. The Phytochemicals present in essential oils are advantageous due to their eco-safety, target-specificity, non-development of resistance, reduced number of application, and higher acceptability with low or no mammalian toxicity. However, the field application of essential oils is hindered due to their low chemical stability and low solubility in water, and suitable formulations are needed to maximize their potential as insecticides [21-24]. To overcome these shortcomings, essential oils can be formulated as seed treatment systems[15-16].

Trifloxystrobin is the methyl ester of (2E)-(methoxyimino) [2-({[(E)-{1-[3-(trifluoromethyl) phenyl]ethylidene}amino] oxy}methyl) phenyl]acetic acid. Trifloxystrobin is a new broad spectrum fungicide. The mode of action of Trifloxystrobin involves inhibition of mitochondrial respiration by blocking electron transfer in the electron transfer chain. A foliar applied fungicide for cereals which is particularly active against Ascomycetes, Deuteromycetes and Oomycetes.

Cashew Nut Shell Oil (CNSO) is a versatile component of the Cashew fruits' nut. The oil which is a dark reddish-brown in colour is resident in a soft honeycomb shell, that is the pericarp of the nut. Cashew nut shell, a by-product of the cashew industry, is an embodiment of a useful chemical serving as a raw material for the petrochemical industry. Cashew (Anacardium Occidentale L.), a well-known species of the Anacardiceae family , is a tropical plant (shrub) found within the region between 23°N and 23°S of the equator.

(Karanj oil) Pongamia oil is derived from the seeds of the Millettia pinnata tree, which is native to tropical and temperate Asia. Millettia pinnata, also known as Pongamia pinnata or Pongamia glabra, is common throughout Asia and thus has many different names in different languages, many of which have come to be used in English to describe the seed oil derived from M. pinnata. It has a high content of triglycerides, and its disagreeable taste and odour are due to bitter flavonoid constituents including karanjin, pongamol, tannin and karanjachromene.

Fungicidal seed treatment formulations offer several benefits including improved seed emergence, plant vigor, and protection against seed and soil-borne fungus. The small size and better coating properties of these formulations enhance stability to droplet aggregation and gravitational separation, as well as their deposition, diffusion, and permeability on pest bodies, leading to enhanced activity of active substances [17-18]. Seed treatment is commonly performed before sowing using seed dressing, seed coating, or seed pelleting. Seed coating involves coating seeds with dry or wet crop protection formulations containing fungicides and/or insecticides. Seed treatment can also be done at harvest to maintain seed quality during storage and transportation [19-20].

In the present study we report novel seed treatment formulations comprising Trifloxystrobin, Cashew nut oil and Karanj oil and evaluate their synergistic effect on crop yield and germination under both lab and field conditions on groundnut crop.

Experimental

2. Material and Method-Stability Study

Chemicals and reagents: Chemicals and reagents required for the study were purchased from Merck India limited, Qualigens Fine Chemicals and SD Fine Chemicals. Potato Dextrose Agar (PDA) used for antifungal activity was procured from Hi Media Lab. Solvents used in saponification, crystallization and column chromatography were laboratory grade. Solvents required for HPLC, GC and MS were analytical grade. Karanj Oil has been purchased from Vijaya Agro Industries Plot No.136, A Section, Sangamner Co-op. Industrial Estate, Tal-Sangamner, Dist-Ahmednagar and Cashew Nut Oil has been procured from Cardolite Corporation, USA.

Instruments: High performance liquid chromatography (HPLC)**:** Analytical reverse phase HPLC (Agilent Infinity II) separation module auto-sampler fitted with controller Open lab CDS software, PDA 1260 DAD WR detector was used for all the analysis. Reverse phase C18 (250 mm × 4.6 mm × 5 µm) analytical column used for the analysis; Malvern Zeta Potential Analyzer to measure particle size of Seed treatment formulation; Eutech pH 700 or TOSHCON PH Meter: to measure pH of formulation before and after stability studies for checking the performance of active ingredients; Newtronic Lab Incubators Model NLLI7SI Cap**:** for shelf-life study of formulations; Kwoya, Model DY300 -Automatic Surface Tensiometer for measuring surface tension of the formulation; Malvern Particle size analyser 3000E was used to measure particle size of formulations; Preparation of nano-emulsion: by IKA-T18 basic, Ultra-TRUX or Silverson L5M and Mettler Toldeo weighing balance; Preparation of Seed Coating Formulation: by IKA-T18 basic, Ultra-TRUX or Silverson L5M, Dyno Mill Research Lab with 80 ml chamber, SATEC Concept ML 2000 and Mettler Toldeo weighing balance.

Preparation of Formulation:
The ten different formulations were prepared using concentrations specified in Table 1. To prepare the gum solution, take the required amount of water, biocide, and defoamer, and homogenize the mixture. Slowly add gum powder while stirring until it dissolves completely. Charge the designated vessel with the required quantity of DM water to produce Flowable concentrate/ Suspension concentrate/ Flowable slurry production. Add the required amount of wetting agent, dispersing agent, suspending agents, colorant/dye to the vessel and homogenize the mixture for 45-60 minutes using a high shear homogenizer. Next, add technical and other remaining adjuvants, excluding antifreeze and thickeners, and homogenize to obtain a uniform slurry ready for grinding. Before grinding, add half the quantity of antifoam and subject it to three cycles of grinding in a Dyno mill. Then, add the remaining half quantity of antifoam along with the antifreeze before sampling for in-process analysis. Finally, add the gum solution to the formulation and stir it for an additional 30 minutes.

Table 1: Preparation of Primary Seed treatment formulation of Trifloxystrobin 3.5%+ Cashew nut oil 15.0 %+ Karanj oil 22.5 % FS

Composition	T-1	T-2	T-3	T-4	T-5	**T-6**	T-7	T-8	T-9	T-10
Karanj oil	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5

Cashew Nut oil	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Trifloxystrobin	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Ethoxylated Fatty Alcohol (wetting agent 1)	1.00	2.00	2.25	2.50	2.50	3.00	3.50	3.75	3.00	3.00
Nonionic polyalkylene glycol ether (wetting agent 2)	0	2.0	2.0	0	2.0	3.0	2.5	0	0	0
Acrylic graft copolymer (dispersing agent 1)	3.0	3.0	3.0	3.0	3.5	0.0	3.0	3.0	3.0	3.0
Alkylated naphthalene sulfonate, sodium salt (dispersing agent 2)	0.10	0.25	0.35	0.45	0.50	0.50	0	0.5	0	0
Sodium polycarboxylate (dispersing agent 3)	0	0	0	0	0	0	3.5	3.5	3.5	3.5
Bentonite Clay (suspending agent)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Silicone antifoam (antifoam)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
1,2-benzisothiazolin-3(2H)-one (preservative)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Glycol (antifreezing agent)	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Polysaccharides (thickener)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Azo dye (colorant)	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
D.M. Water (carrier)	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs
TOTAL	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Field Experiment

Bio-efficacy studies of the trial 6 seed treatment fungicidal formulation were carried out in groundnut crops. Seed treatment fungicidal formulation comprising Trifloxystrobin 3.5%+ Cashew nut oil 15.0%+ Karanj oil 22.5% was developed and evaluated for their efficacy against insect-pest and diseases, effect on growth and vigor of plants, germinations, impact on yield and yield attributing characters.

Experiment was conducted on Groundnut (Arachis hypogaea) to study the synergism between Trifloxystrobin, Cashew nut oil and one more fungicide selected from Karanj oil. The Groundnut seeds are treated with various composition as below:

1. Trifloxystrobin 3.5% + Cashew nut oil 15%+Karanj oil 22.5% FS
2. Trifloxystrobin 3.5% + Cashew nut oil 15% FS
3. Trifloxystrobin 3.5% + Karanj oil 22.5% FS
4. Cashew nut oil 15% + Karanj oil 22.5% FS
5. Trifloxystrobin 3.5% FS
6. Cashew nut oil 15% FS
7. Karanj oil 22.5% FS

The treated seed were sown in the field, filled with sick soil, inoculated with soil borne fungus, Rhizoctonia spp. and or Phytophthora spp. and or Sclerotium spp. The groundnut seeds were also inoculated with Aspergillus niger before seed treatment. 100 seeds were sown per pot and 4 replications per treatment. The observations on seed germination were recorded after two weeks.

3. Results and Discussion

The ten formulations of various composition comprising Trifloxystrobin 3.5% + Cashew nut oil 15%+Karanj oil 22.5% FS tested for their stability in lab and field.

Stability Analysis

Stability analysis of seed treatment fungicidal formulation was performed at three different temperatures: room temperature, 0°C degree and 54°C for 14 days (Table 2 and Fig1). We found that trial 6 (refer Table 1) was more stable on both temperatures due to the perfect combination of Ethoxylated Fatty Alcohol, Nonionic polyalkylene glycol ether and Alkylated naphthalene sulfonate, sodium salt and other related ingredients.

Figure 1: Stability Analysis of various compositions of the formulation at (a) room temperature and (b) 54 ^{0}C temperature (for 14 days).

The stability of seed treatment fungicidal formulation was due to the surfactant used (Ethoxylated Fatty Alcohol, Nonionic polyalkylene glycol ether and Alkylated naphthalene sulfonate, sodium salt), which reduced the interfacial free energy providing the mechanical barrier to calescence [25].

Table 2: Storage stability Study of Trifloxystrobin 3.5% + Cashew nut oil 15%+Karanj oil 22.5% FS

Parameters	**Specification**	**Initial (Ambient temperature i.e RT)**	**Heat stability study at 54 $\pm$ 2 ^{0}C for 14 days**	**Cold storage stability at 0 $\pm$ 2 ^{0}C for 14 days**
Description	Red colored liquid	Red colored liquid	Red colored liquid	Red colored liquid
Trifloxystrobin Content	3.325-3.85	3.88	3.79	3.86
Cashew nut oil content	14.25-15.75	15.39	15.32	15.40
Karanj oil Content	21.375-23.625	22.69	22.60	22.67
Trifloxystrobin Suspensibility	Mini 80%	97	95	97
Cashew nut oil Suspensibility	Mini 80%	98	96	97
Karanj oil Suspensibility	Mini 80%	97	96	97
pH	5.0-9.0	6.5	6.6	6.5
Particle size	D50 <5, D90 <12	3.61, 7.98	3.63, 8.1	3.63, 8.1
Pourability	95 % min	97	96	97
Specific gravity	1.05 – 1.15	1.12	1.12	1.12
Viscosity	350 -900 cps	500	510	530

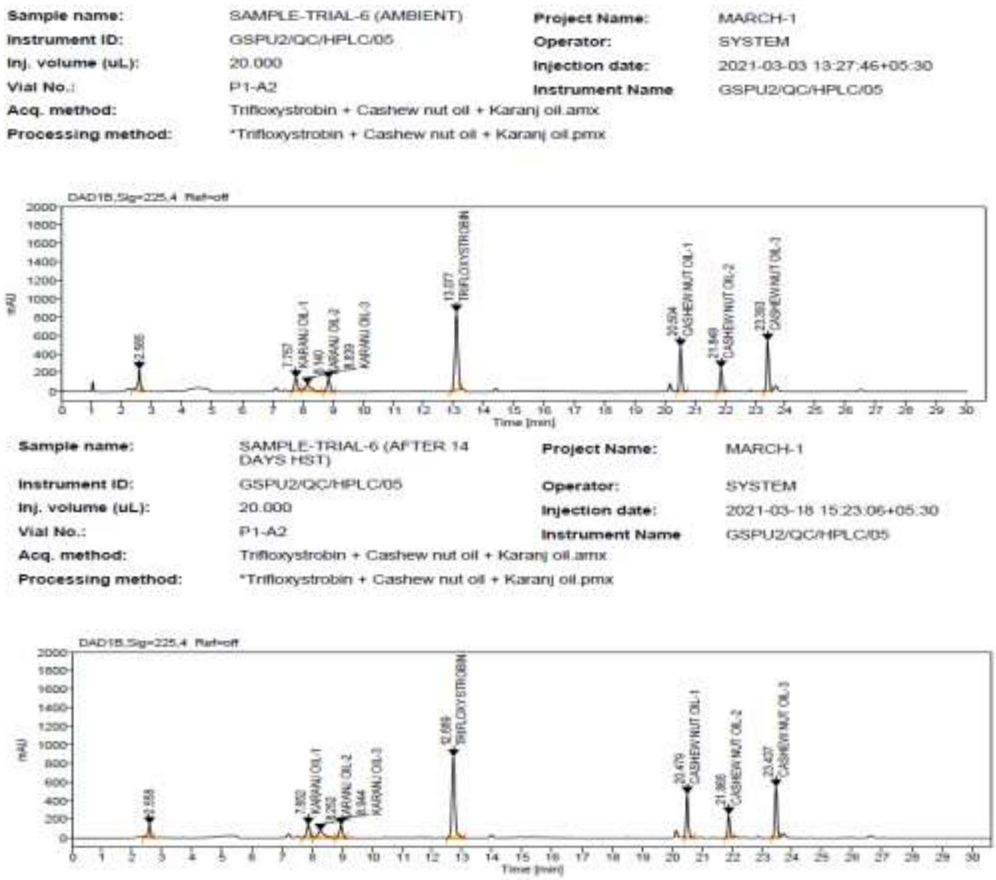
We also studied the degradation of active ingredients in trial 6 under the accelerated testing conditions using HPLC Studies (Table 3 and Figure2). We found there was negligible degradation of active ingredients under these conditions, indicating towards better stability due to the surfactants used in preparation for formulation.

Table 3: Active calculation of Trifloxystrobin 3.5% + Cashew nut oil 15%+Karanj oil 22.5% FS based on HPLC data of sample stored at room temperature.

W/W	**KARANJ OIL 22.5%**	**CASHEW NUT OIL 15.0%**	**TRIFLOXYSTROBIN 3.5%**
SAMPLE AREA	1004.75	2443.63	5866.01

SAMPLE AREA	810.08	1206.45	
SAMPLE AREA	941.3	2899.86	
SAMPLE TOTAL	2756.13	6549.94	
STD WT.	0.1154	0.0658	0.0205
STD.AI.	99	99	96.1
STD AREA	1167.74	2329.31	6769.31
STD AREA	992.75	1153.98	
STD AREA	990.58	2813.54	
STD TOTAL	3151.07	6296.83	
SAMPLE WT	0.4404	0.4404	0.4404
SAMPLE AI.	**22.69**	**15.39**	**3.88**

Figure 2: HPLC analysis at (a) room temperature and (b) 54^{0}C temperature (for 14 days).



The stability of active content of seed treatment fungicidal formulation (trial 6) was analysed using HPLC (Table 4) for ambient and 54^0C samples to check the active content degradation pattern and it was found to have active content in stable range as per our decided concentration (Trifloxystrobin 3.5% + Cashew nut oil 15%+Karanj oil 22.5%). No other trials were analyzed for degradation studies as they were failing in physical stability parameters after 54^0C study for 14 days.

Table 4: Active calculation based on HPLC data of sample stored after 14 days at 54° C

	KARANJ OIL	**CASHEW NUT OIL**	**TRIFLOXYSTROBIN**
SAMPLE AREA	983.74	2419.2	6245.54
SAMPLE AREA	1009.17	1192.2	
SAMPLE AREA	962.79	3005.26	
SAMPLE TOTAL	2955.70	6616.66	
STD WT.	0.1011	0.0677	0.0178
STD.AI.	99	99	96.1
STD AREA	978.03	2375.38	6277.23
STD AREA	975.97	1166.89	
STD AREA	961.52	2906.06	
STD TOTAL	2915.52	6448.33	
SAMPLE WT,	0.4489	0.4489	0.4489
SAMPLE AI.	**22.60**	**15.32**	**3.79**

We further studied the physio-chemical stability of the trail 6 for 24 months (Table 5) and found that is stable for the 24 months and match standards of central insecticide board and registration committee for stability of commercial formulations.

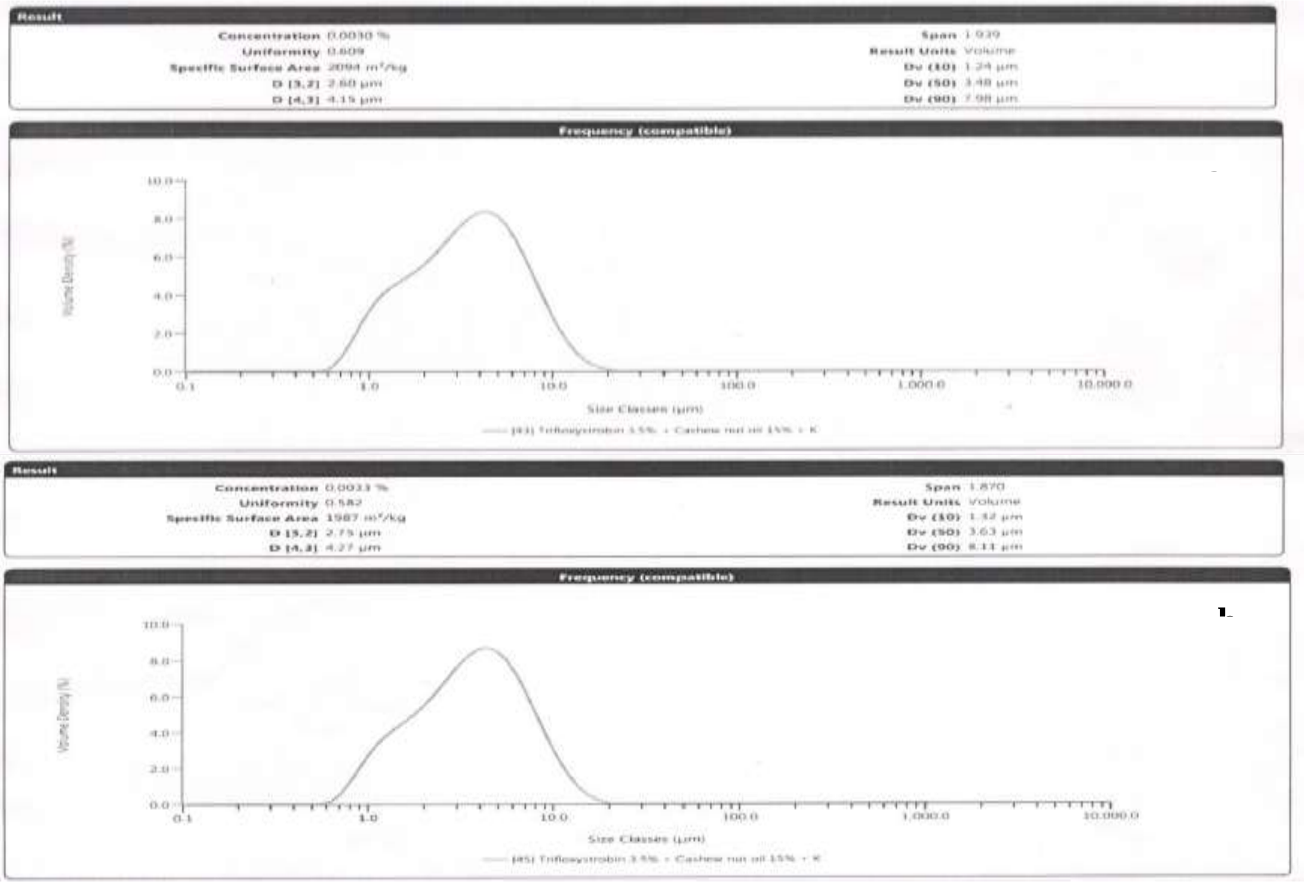
Table 5: Room Temperature storage data of Trifloxystrobin 3.5% + Cashew nut oil 15%+Karanj oil 22.5% FS

Parameters	**Specification**	**Study Duration**			
	In House	**1 month**	**6 months**	**12 months**	**24 months**
Description	colored liquid	Complies	Complies	Complies	Complies
Trifloxystrobin Content	3.325-3.85	3.88	3.80	3.7	3.79
Cashew nut oil content	14.25-15.75	15.39	15.35	15.35	15.32
Karanj oil Content	21.375-23.625	22.69	22.65	22.65	22.60
Trifloxystrobin Suspensibility	Mini 80%	97	97	97	96
Cashew nut oil Suspensibility	Mini 80%	98	97	97	96
Karanj oil Suspensibility	Mini 80%	97	96	97	96
pH	5.0-9.0	6.5	6.5	6.55	6.6
Particle size	D50 <5, D90 <12	3.61, 7.98	3.61, 7.98	3.63, 8.1	3.63, 8.1
Pourability	95 % min	97	97	97	96
Specific gravity	1.05 – 1.15	1.1	1.1	1.1	1.1
Viscosity	350 -900 cps	500	500	500	510

Determination of particle size of Trifloxystrobin 3.5% + Cashew nut oil 15%+Karanj oil 22.5% FS fungicidal combination

We also measured the particle size of formulation using dynamic light scattering (DLS) studies. The DLS measurement was carried out at 25ºC. The sample was prepared in distilled water. 5 mL of the sample solution is taken into vial. The average particle size of this seed treatment formulation with surfactants ranged from 3.50 ±0.5 nm to 5.00±0.5 (Fig. 3).

Figure 3: Particle size Analysis of formulation at (a) room temperature and (b) 54^0C temperature (for 14 days).



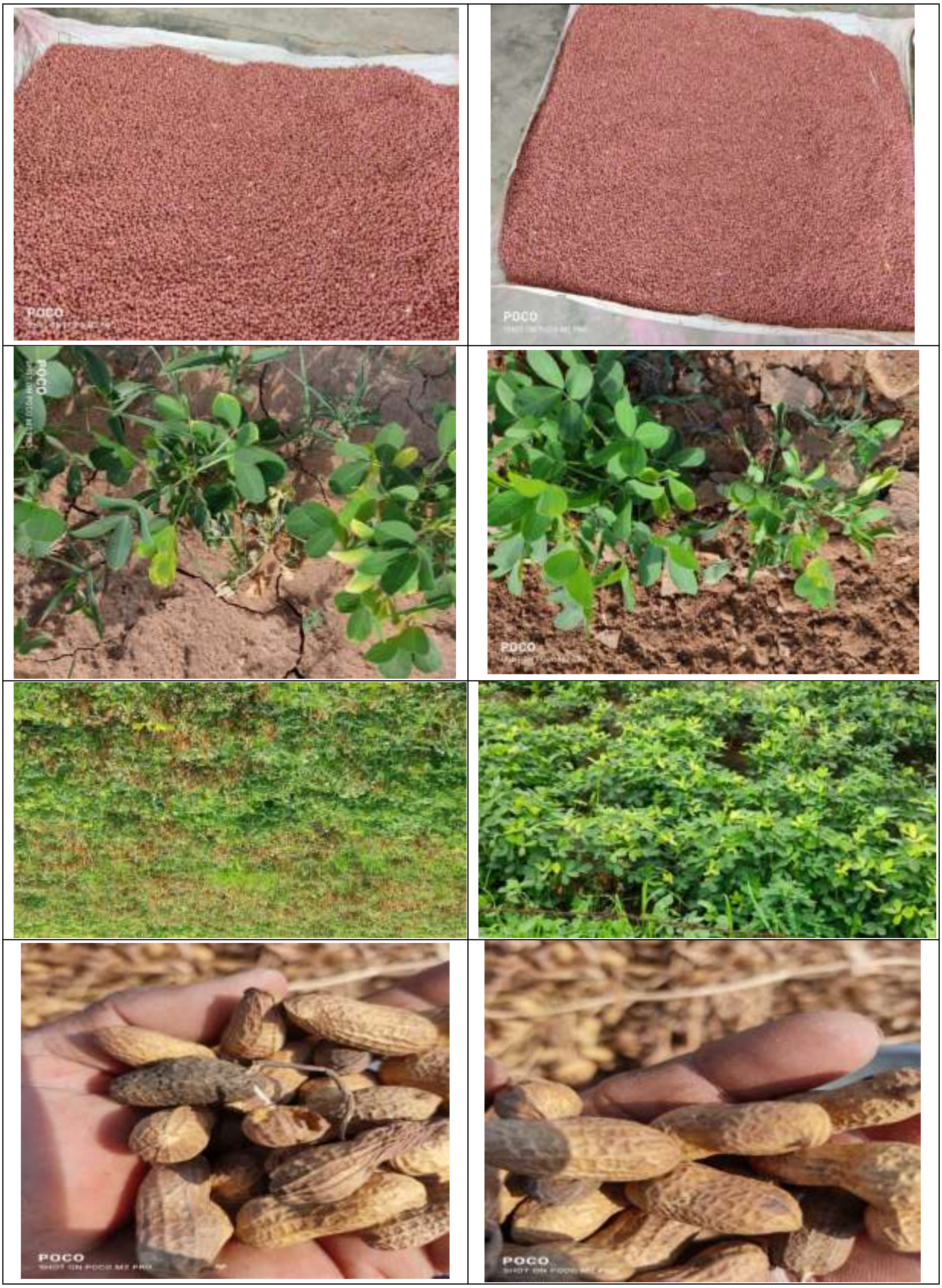
oils have been found to have significant advantages in controlling fungal infections. Due to their small size, they can release the active ingredients slowly and gradually, providing long-lasting effects and better control over the fungal infection. This is because the essential oils can easily penetrate the cell walls of the fungus and disrupt its metabolic pathways, ultimately leading to its death. In addition, the large surface area to volume ratio of nanoparticles increases their contact with the fungus, thereby enhancing their ability to inhibit fungal growth. This property of essential oil nanoparticles makes them a promising alternative to traditional fungicides in agriculture and plant protection. Encouraged by the better stability and small particle size of active ingredients in trial 6 we evaluated its activity in field conditions [28-29].

Effect of Formulation on seed germination and yield

The observations on seed germination shows that synergistic activity observed between Trifloxystrobin, Cashew nut oil and Karanj oil. This seed treatment fungicidal formulation provides good control on soil borne fungus which causes pre-emergence and post emergence seedling rot in groundnut (Fig 4).

Figure 4: Effect of formulation on different stages on crop and crop yield (a) Untreated (b) Treated.

Untreated	**Treated**



The seed treatment fungicidal formulation of Trifloxystrobin 3.5%+Cashew nut oil 15%+Karanj oil 22.5% FS showed 95% groundnut germination, 2893 Kg/hac Haulm yield, 1795 Kg/hac Pod yield and 1292 Kg/hac seed yield which was highest among all the tested combinations (Table 6).

Table 6. Effect of Fungicide Formulation on seed germination and yield

Treatments	Rate per 10 kg seed	% Groundnut germination	Haulm yield (Kg/hac)	Pod Yield (Kg/hac)	Seed yield (Kg/hac)
Trifloxystrobin 3.5%+Cashew nut oil 15%+Karanj oil 22.5% FS	70	95	2893	1795	1292
Trifloxystrobin 3.5%+ Cashew nut oil 15% FS	70	66	2500	1480	1100
Trifloxystrobin 3.5%+Karanj oil 22.5% FS	70	75	2257	1289	1000
Cashew nut oil 15%+Karanj oil 22.5% FS	70	58	2479	1251	1100
Trifloxystrobin 3.5% FS	70	62	1900	1189	978
Cashew nut oil 15% FS	70	33	1985	1214	992
Karanj oil 22.5% FS	70	40	2012	1321	1000
No Fungicide	-	50	1732	953	548

4. Conclusion

In conclusion, the present study has demonstrated the efficacy of a synergistic seed treatment formulation containing Trifloxystrobin, Cashew nut oil, and Karanj oil in controlling soil-borne fungus in Groundnut. The formulation exhibited remarkable stability over a period of 24 months and proved to be highly effective in promoting groundnut germination and yield. The combination of Trifloxystrobin 3.5%+Cashew nut oil 15%+Karanj oil 22.5% FS was found to be the most effective among all the tested combinations, providing 95% groundnut germination, 2893 Kg/hac Haulm yield, 1795 Kg/hac Pod yield, and 1292 Kg/hac seed yield. The use of essential oils with Strobilurin fungicide in the right ratio and seed treatment technique can reduce the reliance on chemical-based pesticides, thus minimizing human exposure to residual chemicals in food commodities. Therefore, seed treatment can be considered a crucial component of sustainable crop production, and its potential for agricultural application is vast.

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