Original Research Article

EVALUATION OF STORAGE TECHNIQUES TO IMPROVE THE SEED LONGEVITY AND SHELF LIFE OF HORSEGRAM.

UNDER PEER REVIEW

ABSTRACT

Horsegram (*Macrotyloma uniflorum*) is a robust, drought-resistant crop that is valued for its high protein content and is a significant food source in many parts of the world. Horsegram seeds must be stored properly to preserve their freshness, nutritional value, and viability for future planting. In this article an investigation was carried out to evaluate the suitable seed treatment and packaging materials for maintaining the shelf life of horsegram seeds during storage.The laboratory experiment was carried out at Department of Seed Science and Technology, Agricultural College andResearch Institute, Tamil Nadu Agricultural University, Madurai, India. The graded seeds of horsegram were treated with different botanicals (Neem seed kernel powder @ 5g/kg &Pungam leaf powder @ 5g/kg), herbal oil (Neem oil @ 5ml/kg) and chemical (Malathion @ 5g/kg) and packed in cloth and super grain bags which was kept under ambient condition. The physical, physiological and biochemical parameters were assessed from the stored seed samples.Horsegram seeds dusted were malathion @ 5g/kg and then packed with super grain bag have better germination (94 %), root length (18.33 cm), shoot length (9.10 cm), DMP (0.165 g seedlings^{-10}), vigour index I & II (2551 & 16.2) and also biochemical parameters after the storage of 8 months. Conclusion?

KEYWORDS: Horsegram, seed storage, treatments, packaging materials, germination

Introduction

Comment [ME1]: Provide background study of using botanicals, malathion in the research. Please write an innovation of the research.

Horsegram(*Macrotylomauniflorum*)belongs to family Fabaceae. It is a potential grain legume having excellent nutritional and remedial properties with better climate resilience to adapt harsh environmental conditions. It is one of the most important unexploited food legume being grown in almost all over the world including temperate and sub-tropical regions. In India, horsegram is cultivated in 4.61 lakh ha with productivity of 1000 kg/ha in Telangana followed by Uttarkhand with a productivity of 923 kg/ha. In Tamil Nadu, horsegram is cultivated in 0.8 lakh hawithproductivityof691kg/ha (Indiastat, 2019). Seed is an important input in agriculture that impacts not only production but also productivity, therefore maintaining seed quality and vigour during storage is crucial. Storage is a basic technique in the control of the physiological quality of the seed, and it is a way for preserving the viability of the seeds and maintaining their vigour during the time between planting and harvesting. Seed storage is necessary to preserve high seed quality for future use. Packing of seeds is done to prevent the absorption of water from atmosphere after drying and also minimize the contamination of seeds due to insects and diseases. Seeds are stored in moisture proof containers removed the dampness, deterioration, microbes and maintain the seed longevity in brinjal seeds (Kumar *et al.* 2008).Sunflower seeds in kraft paper packing were stored more effectively and maintained physiological integrity in a cold chamber (Abreu *et al.* 2013). Seed treatments play a significant role in improving the establishment of healthy crops as well as reduce losses of seed during storage. Soyabean seeds treated with Imidacloprid and Tiocarbe + Carbendazim and Tiram maintained the physiological quality of seeds stored for a period of two months (Ferreira *et al.* 2016). Keeping this background, present study was~~is~~ carried out to identify the suitable seed treatments and packing material to maintain the shelf life of horse gram seeds.

Materials and Methods

The graded seeds of horsegram were treated with different botanicals, herbal oil and chemical as per the treatment scheduled*viz*, T_0 -Control, T_1-Malathion @ 5g/kg, T_2-Pungam leaf powder @ 5g/kg, T_3-Neem oil @ 5ml/kg,T_4-Neem seed kernel powder @ 5g/kg. The treated seeds were allowed to dry back to their original moisture content, packed in cloth (C_1) and super grain bags (C_2) which was kept under ambient condition for about eight months by following FCRD. The seed samples were drawn bimonthly interval and subjected to quality analysis *viz.,*seed physical character Seed Moisture Content (%) and physiologicalcharacters such asgermination (%), root length (cm), shoot length (cm), dry matter production (g/10 seedlings) and vigour index (Abdul- Baki and Anderson, 1973).Seed moisture content was calculated and the mean expressed as percentage adopting the following formula.

$$\text{Moisture content (\%)} = \frac{M2 - M3}{M2 - M1} \times 100$$

Comment [ME2]: Where were to get the samples and all the materials used?

UNDER PEER REVIEW

Where,

M1=Weight of the empty container with its cover, M2= Weight of the container with its cover and seeds before drying.

M3= Weight of the container with its cover and seeds after drying.

The germination test was carried out as per the procedure prescribed by ISTA (2013). After nine days, the normal seedlings produced were counted and expressed as percentage.The vigour index I and vigour index II were calculated using following formula.

Vigour index I = Germination (%) × Total seedling length (cm).

Vigour index II = Germination (%) x Dry matter production (g/10 seedlings).

Further, the seed biochemical parameters like Dehydrogenase (OD value) activity (Kittock and Law, 1968) , Electrical conductivity (dSm^{-1}) (Presley, 1958), Protein content (%) (Ali-Khan and Youngs, 1973),Catalase activity (Units g^{-1})(Luck, 1974) were also estimated.From the optical density values, the protein content was calculated using the following formula.

$$\text{Protein content (\%)} = 3.78 + (61.6 \times \text{Optical Density value})$$

Catalase activity was expressed as units/g tissue using the following formula.

$$\text{Catalase activity} = \frac{17 \times 10 \times 20 \times 1000}{\Delta t \times X \times Y} \times 100$$

Where

Δt – Time required todecrease the absorbance;X – Volume of enzyme extract

Y – Volume of buffer solution

Seed infestation was indicated through presence of holes in each treatement counted and expressed in percentage.

$$\text{Per cent Infestation (\%)} = \frac{\text{No. of damaged seeds}}{\text{Total number of examined seeds}} \times 100$$

UNDER PEER REVIEW

STATISTICAL ANALYSIS

The data obtained from different experiments were analysed by the ‘F’ test of significance following the methods described by Panse and Sukhatme (1978). Wherever necessary, the per cent values were transformed to angular (Arc-sine) values before analysis. The Critical Differences (CD) was calculated at 5 per cent probability level. The data were tested for statistical significance. If the F test is non-significant it was indicated by the letters NS.

RESULT AND DISCUSSION

Seed storage is an important division of seed industry. In storage, vigour and viability of the seeds are maintained by many physic-chemical factors like initial seed quality, physical and chemical composition of seed, moisture content of seed, temperature, storage structure, gaseous exchange and packaging materials. Seed is hygroscopic in nature, storage environment act as a main role on the variation in moisture content {(Padma and Muralimohan Reddy (2001); McDonald (2004); Lehner *et al.* (2008)}. Seed is a biotic unit and the seed cells are made up of lipoprotein layers, they gain moisture during storage then fasten the lipid per-oxidation activity that leads to deterioration and at last death of the seed (Agarwal and Dadlani, 1995). In the current study, the seeds were treated with botanicals and chemical compounds before storage and packed in two containers like cloth bag and super grain bag evaluated for their suitability.

Seed physical characters during storage

Seed quality during storage can be governed by crucial factors such as the moisture content of the seed, storage conditions and packing materials. When seeds have been dried to a moisture level that is in equilibrium with 5–20°C and 10–25% relative humidity, seed longevity during storage will be maximised (FAO, 2013).In this study, moisture content was increased in stored seeds especially in cloth bag (8.3%) than super grain bag (7.6%) at the end of 8 month of storage period.Initially, the moisture content was 7.3 %. Because cloth bag wasis moisture pervious container and it permits the exchange of moisture in seed with environment. Minimum variation of moisture content was observed in super grain bag stored seeds, it’s due to its impervious in nature and offered protection to seeds.Rise in seed moisture wasis due to the hygroscopic nature of seed (Harrington, 1972; Roberts, 1989). Minimum absorption of moisture was

UNDER PEER REVIEW

taken place in super grain bag after 8 months in maize seeds and also registered the lowest oxygen content opined by Anuradha Kumari *et al.* (2017). This was supported with Pundlik (2015) in paddy; Umesha *et al.* (2017) and Beedi *et al.* (2018) in chickpea.

Seed physiological characters during storage

Germination capacity is the basic requirement for seeds. Initially, 100 % of germination was obtained and it was declined to 89%irrespective of seed treatments and containers. The observation of other parameters *viz.,* root length, shoot length, drymatterproduction andvigour index also declined with increase of storage period. At the end of storage period, seeds packed in super grain bag hadve the highest germination percentage (91 %) compared to cloth bag (86 %) **(Plate.1) (Table 1)**. On the other hand, the seeds in super grain bag registered the minimum reduction in all vigour parameters such as root length (18.0cm),shoot length (8.76 cm), drymatter production (0.161 g seedling^{-10}) and vigour index I (2436) & II (14.7) **(Table 2&3)**while in cloth bag stored seeds recorded drastic reduction in vigour parameters.Seeds stored in Triple layer plastic bags showed higher germination percentage and other physiological characters because it prevented major damage caused by bruchids (*Callosobruchus maculatus* F.) in cowpea seeds, while seeds stored in gunny bags suffered severe losses (Vales *et al.,* 2014).Seedling vigour was higher in super grain stored seeds is due to the lowest seeddamage (Kalsa *et al.,* 2019).

UNDER PEER REVIEW

Table 1: Effect of seed treatment, storage containers and period of storage on germination (%) of horse gram

Treatment (T)	Containers (C) and Period of storage (P)												Grand Mean
	Cloth bag (C_1)						Super grain bag (C_2)						
	P_0	**P_2**	**P_4**	**P_6**	**P_8**	**Mean**	**P_0**	**P_2**	**P_4**	**P_6**	**P_8**	**Mean**	
T_0	100 (89.72)	93 (74.66)	90 (71.56)	87 (68.86)	84 (66.42)	**91 (72.54)**	100 (89.72)	97 (80.03)	94 (75.82)	91 (72.54)	89 (70.63)	**94 (75.82)**	**93 (74.66)**
T_1	100 (89.72)	98 (81.87)	95 (77.08)	91 (72.54)	88 (69.73)	**94 (75.82)**	100 (89.72)	100 (89.72)	98 (81.87)	96 (78.46)	94 (75.82)	**98 (81.87)**	**96 (78.46)**
T_2	100	94	91	89	85	**92**	100	98	95	93	90	**95**	**94**

	(89.72)	(75.82)	(72.54)	(70.63)	(67.21)	**(73.57)**	(89.72)	(81.87)	(77.08)	(74.66)	(71.56)	**(77.08)**	**(75.82)**
T_3	100 (89.72)	96 (78.46)	93 (74.66)	91 (72.54)	87 (68.86)	**93 (74.66)**	100 (89.72)	100 (89.72)	97 (80.03)	94 (75.82)	92 (73.57)	**97 (80.03)**	**95 (77.08)**
T_4	100 (89.72)	95 (77.08)	92 (73.57)	90 (71.56)	86 (68.03)	**93 (74.66)**	100 (89.72)	99 (84.26)	96 (78.46)	93 (74.66)	91 (72.54)	**96 (78.46)**	**94 (75.82)**
Mean	**100 (89.72)**	**97 (80.03)**	**92 (73.57)**	**89 (70.63)**	**86 (68.03)**	**93 (74.66)**	**100 (89.72)**	**99 (84.26)**	**96 (78.46)**	**93 (74.66)**	**91 (72.54)**	**96 (78.46)**	**94 (75.82)**

	T	**P**	**C**	**TXP**	**PXC**	**TXC**	**TXPXC**
S. Ed	0.26	0.25	0.16	0.57	0.36	0.35	0.80
CD (P=0.05)	0.51	0.50	0.32	1.13	0.71	NS	NS

(Values in parentheses indicate arcsine transformed values);

C- Containers; P- Storage period in months; T- Treatments

T_0- Control **T_1-** Malathion 5g/kg **T_2-**Pungam leaf powder 5g/kg

T_3- Neem oil 5ml/kg **T_4-**Neem seed kernel powder 5g/kg

Table 2: Effect of seed treatment, storage containers and period of storage on vigour index I of horse gram

Treatment (T)	**Containers (C) and Period of storage (P)**												**Grand Mean**
	Cloth bag (C_1)						**Super grain bag (C_2)**						
	P_0	**P_2**	**P_4**	**P_6**	**P_8**	**Mean**	**P_0**	**P_2**	**P_4**	**P_6**	**P_8**	**Mean**	
T_0	2859	2504	2383	2272	2117	**2427**	2859	2652	2531	2385	2326	**2551**	**2489**

UNDER PEER REVIEW

T_1	2859	2773	2654	2507	2406	**2640**	2859	2847	2752	2671	2551	**2736**	**2688**
T_2	2859	2583	2468	2371	2220	**2500**	2859	2710	2590	2484	2389	**2606**	**2553**
T_3	2859	2675	2565	2470	2324	**2579**	2859	2794	2691	2568	2478	**2678**	**2628**
T_4	2859	2611	2500	2400	2258	**2526**	2859	2752	2627	2504	2435	**2635**	**2581**
Mean	**2859**	**2629**	**2514**	**2404**	**2265**	**2534**	**2859**	**2751**	**2638**	**2522**	**2436**	**2641**	**2588**

	T	**P**	**C**	**TXP**	**PXC**	**TXC**	**TXPXC**
S. Ed	18	18	11	40	25	25	57
CD (P=0.05)	35	35	22	79	50	NS	NS

C- Containers; P- Storage period in months; T- Treatments

T_0- Control **T_1**- Malathion @ 5g/kg **T_2**-Pungam leaf powder @ 5g/kg

T_3- Neem oil @ 5ml/kg **T_4**- Neem seed kernel powder @ 5g/kg

Table 3: Effect of seed treatment, storage containers and period of storage on vigour index II of horse gram

Treatment (T)	**Containers (C) and Period of storage (P)**												**Grand Mean**
	Cloth bag (C_1)						**Super grain bag (C_2)**						
	P_0	**P_2**	**P_4**	**P_6**	**P_8**	**Mean**	**P_0**	**P_2**	**P_4**	**P_6**	**P_8**	**Mean**	
T_0	17.2	15.1	14.3	13.7	12.7	**14.6**	17.2	16.0	15.2	14.4	14.0	**15.3**	**15.0**
T_1	17.2	16.7	16.0	15.1	14.5	**15.9**	17.2	17.1	16.6	16.1	15.4	**16.5**	**16.2**
T_2	17.2	15.5	14.9	14.3	13.4	**15.0**	17.2	16.3	15.6	14.9	14.4	**15.7**	**15.4**
T_3	17.2	16.1	15.4	14.9	14.0	**15.5**	17.2	16.8	16.2	15.4	14.9	**16.1**	**15.8**
T_4	17.2	15.7	15.0	14.4	13.6	**15.2**	17.2	16.6	15.8	15.1	14.7	**15.9**	**15.5**
Mean	**17.2**	**15.8**	**15.1**	**14.5**	**13.6**	**15.2**	**17.2**	**16.6**	**15.9**	**15.2**	**14.7**	**15.9**	**15.6**

	T	**P**	**C**	**TXP**	**PXC**	**TXC**	**TXPXC**
S. Ed	0.1	0.1	0.03	0.1	0.1	0.1	0.2
CD (P=0.05)	0.1	0.1	0.1	0.2	0.2	NS	NS

C- Containers; P- Storage period in months; T- Treatments

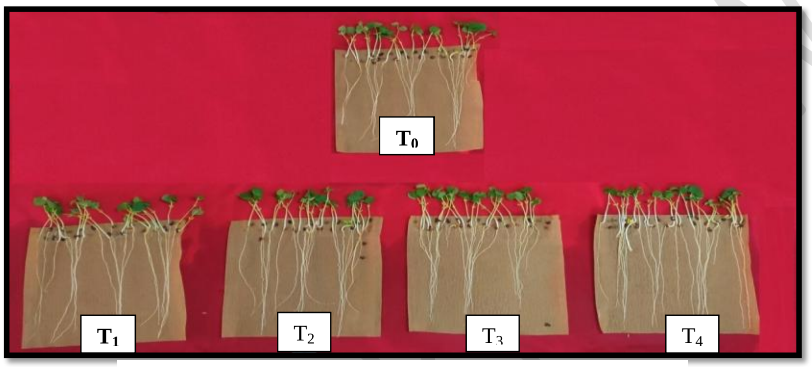
T_0- Control **T_1**- Malathion @ 5g/kg **T_2**-Pungam leaf powder @ 5g/kg

T_3- Neem oil @ 5ml/kg **T_4**-Neem seed kernel powder @ 5g/kg

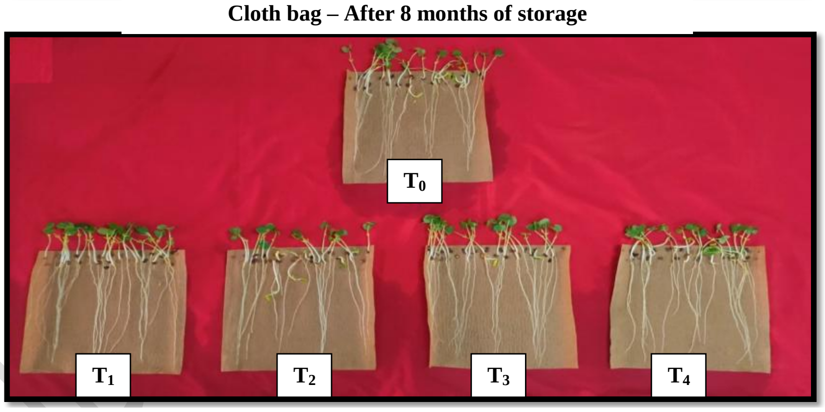
Plate 1: Effect of seed treatments and storage containers after 8 months of storage onhorsegram

UNDER PEER REVIEW

INITIAL



Cloth bag – After 8 months of storage



Super grain bag – After 8 months of storage

T_0-Control **T_1**-Malathion 5g/kg **T_2**-Pungam leaf powder 5g/kg

T_3-Neem oil 5ml/kg **T_4**-Neem seed kernel powder 5g/kg

Imp

1967).Vigour and viability ~~are~~ decreased during storage by many biotic factors like microflora and storage pests. So chemicals and botanicals were~~are~~ used to reduce the qualitative and quantitative losses and also it maintain~~s~~ed the quality of seed for longer period. Among the seed treatments, Malathion treated seeds ha~~d~~ve higher germination (96%) than control (93%) after the storage of 8 months**(Table 1)**. Similar results were reportedbyBahukhandi *et al.* (2017) in

Comment [ME3]: Provide some reasons.

berseem andJagatap *et al.* (2014) inurdbean. Malathion dusted seeds with higher seed quality parameters than control was registered byMalarkodi and Srimathi (2001) in maize and Srimathi *et al.* (2001) in pearlmillet.

The other physiological parameters like shoot length (9.42 cm), root length (18.66 cm), DMP (0.169 g seedling^{-10})and vigour index I (2736) & II (16.5)**(Table 2&3)** were higher in seeds treated with malathion 5 g/kg and packed in super grain bag than control. Similar results were registered in cowpea (Lamani and Deshpande, 2016) andSunhemp (Thimmanna *et al.*, 2014). Malathiontreated seeds were deteriorated slowly due to the phosphate analogue addition to insecticidal property which favours the insect control, reduce deterioration and minimize the role of biotic factors was reported by Eevera (2000) in blackgram. Similar result was supportedby Malarkodi (2003) in green gram.

Seed biochemicalcharacters during storage

The seeds treated with malathion @ 5g/kg and packed in super grain bag registered the lowest electrical conductivity (0.121dSm^{-1})**(Fig.3)**compared to other treatments and control. This might be due to reduced seed permeability thus resulting lower electrical conductivity of leachates. Guha *et al.*, (2012) reported that okra seeds treated with red chilli powder and aspirin show extended protection against cell membrane and repair mechanisms during seed storage.

The protein content (19.21%)**(Fig.4)** and the activity of enzymes like Dehydrogenase (0.521)**(Fig.2)**and catalase(0.213 units g^{-1})**(Fig.1)** were higher in malathion treated seeds which packed in super grain bag at the end of 8 months of storage periodwas registered as an added seed quality which influences the seeds storability in storage. Menaka (2000) reported activities of enzyme are answerable for respiration of seed reduced by age of seed with additive response with the factors accountable fordeterioration. Thimmanna *et al.* (2014) reported that dehydrogenase activity was higher in malathion treated seeds in sunhemp.

Seeds in cloth bags were highly damaged by bruchids and recorded the highest seed infestation (1.92%), compared to super grain bags (0.61%) at the end of 8 month of storage, whereas control bags recorded (2.27%).In super grain bags, the presence of minimum amount of oxygen was consumed by the respiration process of insects and

UNDER PEER REVIEW

microbes, which resulted in a carbon dioxide-rich atmosphere that reduced the insect infestation.Parellel results were investigated by Baldaniya *et al.* (2013); De Groote *et al.* (2013); Bakhtavar *et al.* (2019)andCovele*et al.*, (2020).

CONCLUSION

Thus, the study revealed that among the seed treatment the malathion @5 g/kg treated seeds showed the highest germination percentage, vigour index, and lowest electrical conductivity leachets compared to other botanical seed treatments. Among the packingmaterials, seed stored in super grain bag has superior quality compared to cloth bag storage in terms of the physical, physiological, and biochemical parameters of the seed. Therefore, seeds treated with malathion @5 g/kg and stored in super grain bag are suitable to maintain the viability of seeds during storage in horse gram.

Effect of seed treatment and packaging material on biochemical properties of horsegram

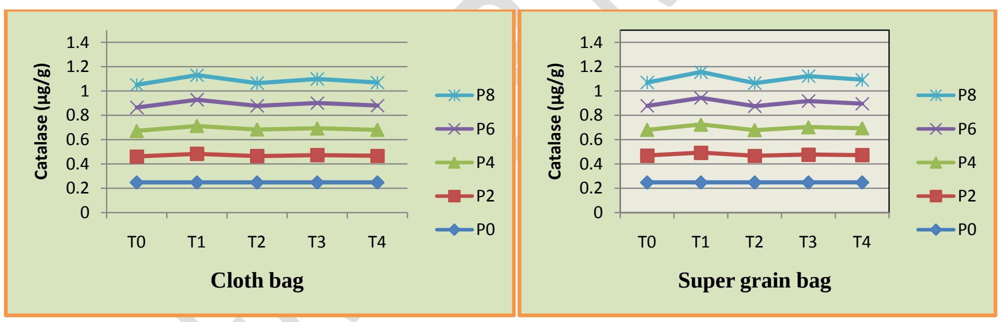
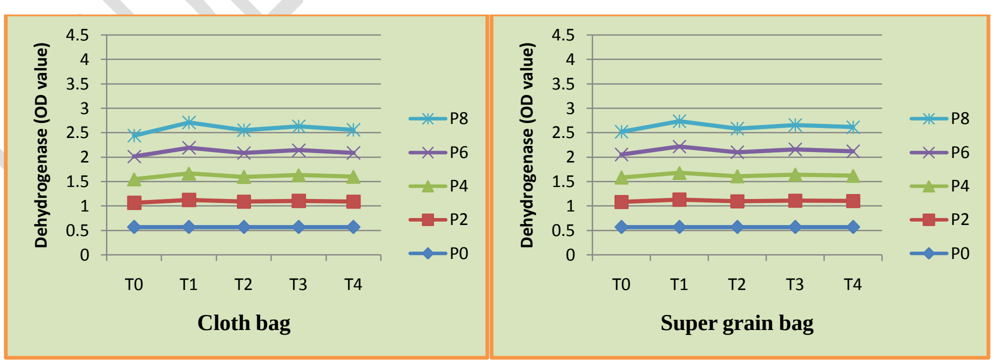


Fig. 1: Catalase activity (µg/g)



UNDER PEER REVIEW

Fig. 2: Dehydrogenase activity (OD value)

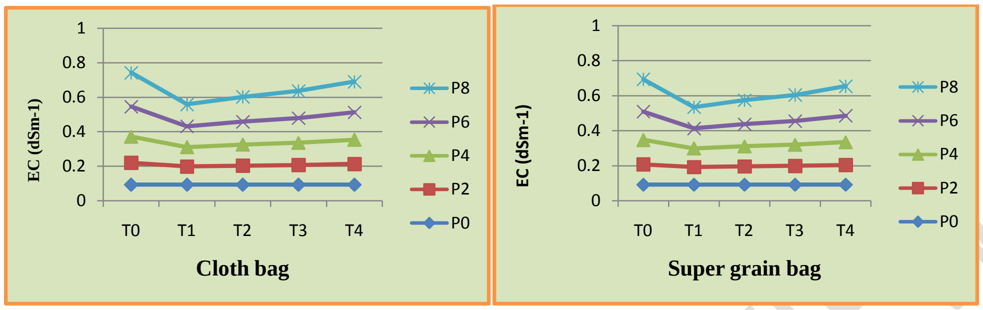


Fig. 3: Electrical conductivity(dSm^{-1})

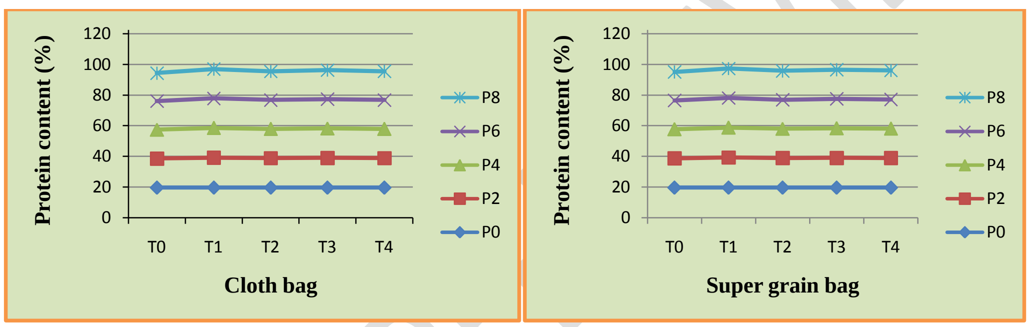


Fig. 4: Protein content (%)

T_0- Control **T_1**- Malathion @ 5g/kg **T_2**- Pungam leaf powder @ 5g/kg

T_3- Neem oil @ 5ml/kg **T_4**-Neem seed kernel powder @ 5g/kg

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