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EVALUATION OF BIOAGENTS AND BIOFERTILIZERS FOR THE MANAGAMENT OF SEED AND SEEDLING DISEASES OF SESAMUM INDICUM (SESAME)

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ABSTRACT

The study was carried out to evaluate the response of biopeticides and biofertilizers on seed mycoflora and seed quality parameters of Sesame (*Sesamum indicum* L.). Untreated Sesame seeds were collected from farmers of Nizamabad and Karimanagar districts of Andhra Pradesh in India and discolored seeds were separated and treated with biofertilizers and biopesticides alone and in combination form. The seed mycoflora of Sesame seeds were screened by using Potato dextrose agar (PDA) medium and czaepek dox agar media. The results indicate that maximum numbers of fungi were recorded on PDA. The untreated seeds were found to be associated with maximum per-cent incidence of mycoflora and minimum population was recorded in the treatment of *Trichoderma* + Pseudomonas formulation followed by *Azat obacter* + *Trichoderma*, Pseudomonas and *Azatobacter* in the decreasing order of efficacy. This study also showed relation of biofertilizers and biopesticides and seed mycoflora on seed germination. Germination percentage was maximum in the treatment *Trichoderma* + Pseudomonas formulation, *Azatobacter* + *Trichoderma*, Pseudomonas and *Azatobacter* in the control, germination percentage was minimum compared with other treatments. Seeds treated with the mixed formulation were found beneficial in reducing the pathogenic fungi and decreasing seedling mortality.

Keywords: Sessame, Biopesticides, Bio-agents, Bio-fertilizers, Seedling decay, Seed borne pathogens.

INTRODUCTION

India, China, Sudan and Mexico are the important sesame (Sesamum indicum L.) producing countries. India ranks first both in area and production of sesame in the world. Sesame is grown on 21 lakh hectares in only eight states covering 2,37,000 in Andhra Pradesh, (Anonymous, 2013). Sesame oil is used as a cooking oil in Southern India. The sesame cake is rich source of protein, carbohydrates and mineral nutrients. Seed borne mycoflora are carried over by infected seeds. They cause deterioration in seed in soil before germination causing seedling mortality. However, Several workers in sesame seeds have indicated the presence other fungi like Alternaria dianthicola, Aspergillus flavus, A. ustus and Macrophomina were detected on sesame. Alternaria sesami, A. sesamicola and A. tenuis. were detected in seeds collected from farmer fields (Enikuomehin, 2010). Similarly, field fungi like Curvularia lunata, fusarium

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moniliforme, were found to isolated from sesame seeds (Abdul and Ansar, 1992). Apart from this, deterioration effect of the fungi like Penicillium rubrum and Rhizopus nigricans (Mbah and Akuishi, 2009) producing toxic substances, the most importantly aflatoxin producing fungi like Aspergillus flavus, A. niger were also reported in Sesame (Alexopolues and Mims, 1979). The seed quality is affected by prevailing environmental conditions by the time seeds reaches physiological maturity from harvest. Seed storage if influenced by temperature and higher relative humidity which tend to deteriorate almost all kinds of seeds rapidly. Storage fungal activity can cause rapid deterioration of seed both in terms of dry matter and quality often loss of nutritive value with decreased viability (Bhattacharya and Raha, 2002).

Seed borne fungal mycoflora are of considerable importance due to their influence on the overall health germination and final crop stand in the field. Infected seeds play a key role in the dissemination of plant pathogens and disease establishment (Raj *et al.*, 2007).

Some of the seed borne fungi were found to be very destructive causing seed rot and obstructing seed germination leading to pre and post emergence seed rot (Sharma, 2010). Fungicides have been reported for many years to control plant pathogens and the use of fungicide seed dressing chemicals bio-agents has become an inevitable method of disease control and pest control in Sesame (Anandu et al., 2010). Seed treatment with bio control agents along developed for control of seedling disease of soybean have been reported both in field and storage Some the potential bio-agents that could be employed for management of seed borne pathogens are T. viride, T. harzianum, Bacillus subtilis and Pseudomonas fluorescens for soybean seed rots (Rajeswari and Meena kumari, 2009). In view of the above, the present investigation was carried out with seed treatment using bio-agents to manage seed and seedling disease of Sesame both in lab and field conditions. Though fungicides are effective (Ashirwar, et al., 2008) in sesame, bio-agents if exploited, will be an economically viable and ecofriendly approach.

MATERIALS AND METHODS

Seed Infection

a. Collection of sample: Twenty seed samples of farmer saved sesame seeds for sowing purpose (locally grown) were collected from different locations of Nizamabad and Karminagar districts of Andhra Pradesh in India. The samples were stored at 5^oC until use.

b. Seed health assay: Seed health assay was carried out using by four methods of seed health testing like Agar plate method, Blotter method, 2,4 D method and Deep freeze method by using sterilized and un sterilized (ISTA, 1996). Seeds prior to plating were surface sterilized in 0.1 percent mercuric chloride solution for 30 to 45 sec followed by 3 to 4 successive washing in sterilized water. Two hundred seeds were randomly taken from each sample. In Blotter method, blotter discs dipped in beaker containing sterile water with the help of forceps and placed at the bottom of each sterilized Petri Plate. 200 seeds from each samples were tested for mycoflora (10 plates @ 20 seeds per each plate). Petri Plates were labeled and incubated at 25± 1°C. These Petri Plate were further incubated at 25±1°C for seven days under 12 h alternating cycles of near UV light and darkness.

In 2 4 dihydroxyphenoxy (2, 4, D) method of testing, seeds were plated on the blotters dipped in 24 D solution and in the deep freeze method similar

procedure of blotter method was followed and the plates were incubated for 24 h at $25\pm1^{\circ}$ C. In deep freeze after planting the seeds, plates were kept at $25\pm1^{\circ}$ C for 24 h and again deep freeze them for 24 h , before final incubation at 21-22° C under cycles of 12 h darkness and 12 h near UV light. These Petri Plates were further incubated at $25\pm1^{\circ}$ C for seven days under 12 h alternating cycles of near UV light and darkness.

After 7 days of incubation under alternating light condition, the seeds were examined for fungal growth under stereomicroscope. Identification of fungi was carried out based on their habit character of fungal growth on seeds and microscopic examinations of conidia following Mathur and Kongsdal (2003). The per cent total number of fungal colonies per 200 seeds was calculated.

c. Detection of seed borne fungi: The seed borne fungi associated with sesame seeds were determined by standard method ie., agar plate method (ISTA, 1999). In this method, potato dextrose agar medium (PDA) was poured aseptically in the sterilized glass petri plates of 9 mm diameter @ about 15 to 20 ml. After 24 h of pouring the medium 15 seeds per petri plate were plated aseptically. Seeds prior to plating were surface sterilized in 0.1 per cent mercuric chloride solution for 30 to 45 sec followed by 3 to 4 successive washing in sterilized water and absorption of excess moisture by keeping the seed on sterilized blotting paper in aseptic conditions. These Petri plates were further incubated at 25± 2°C for seven days under 12 h alternating cycles of near UV light and darkness. These seeds were examined for fungi after five and seven days of incubation. Pure cultures of the fungi associated with seeds were sub-cultured in tubes with the help of sterilized inoculation needle or sterilized forceps in the inoculation chamber. These tubes were incubated at a temperature of $25 \pm 2^{\circ}$ C. Observations on fungal growth were regularly taken and subcultures of fungi were made to get pure cultures. Preliminary identification of the fungi was done on the basis of the colony characters developed on the agar medium and the morphology of the individual fungal species. The identification was done on the basis of the colony characters with the help of manuals on seed borne fungi.

Seed treatments

Seeds collected from the farmers were thoroughly washed with distilled water, air dried. The dried seeds were treated with bio-agents and biofertilizers as per the recommendations. The carrier based inoculum's of biofertilizers and bioagents were obtained from KN Biosciences, Miyapur and Agribiotech foundation, Rajendranagar. These were used singly and in combination ie., Trichoderma viride + Pseudomonas, Azatobacter + Trichoderma, Rhizobium + Trichoderma, Azatobacter, Trichoderma, Pseudomonas, Benomyl and untreated control. Trichoderma and Pseudomonas were treated @ 6g/kg and 10 g/kg seed, respectively. Azatobacter was used @ 25 g/kg seed (250g/10 kg seed). The combination inoculum was used @ half the dose of each bioagent/biofertilizer.

a. Enumeration of total fungal colonies by Blotter method: Discs of blotter paper were kept in 90 mm Petriplates. Blotter discs were wiped in beaker containing sterile distilled water with the help of forceps and placed at the bottom of each sterilized Petri plate. Two hundred seeds from each treatment were tested in 20 plates of 10 seeds per plate. Petriplates were labeled and incubated at 25+ 2°C under alternating cycles of 12 h light and 12 h day for 9 days in Bio chemical Oxygen Demand incubator. The plates were examined under stereo binocular microscope on 9th day of incubation in standard blotter method. Infestations levels were recorded as percentage of infested seeds in each sample. Percent seed rot and seedling decay were recorded from the treated seeds in blotter method.

b. Germination Assay: The treatmental effect on germination of seeds was determined by rolled paper towel method (ISTA 1996) and sand method with some modifications as described below.

i) **Blotter Method**: One hundred seeds in five replications for each treatment were assessed and **germination** tests were conducted using Petriplates with filter paper placed in them. The number of normal seedling were counted at two days interval and cumulative germination obtained on the 8th day was recorded. Per cent seed germination was then expressed as number of seeds germinated over total number of seeds plated.

ii) Paper towel method: Fifty seeds were treated with bio-agents and biofertilizers were placed on two moist paper towels of 23 X 30 cm size per replication. Seed were kept as an appropriate spacing and covered with another moist paper towel and rolled up. The rolled paper towels were kept in an upright position in an incubator at 28 = 20C. The germination counts were taken after 14 days on number of normal seedlings,

abnormal seedlings seed rots and un-germinated seeds. The seeds infested with the fungi were recorded.

iii) Sand method: This experiment was conducted in plastic trays having 45 X 30 X 8 cm size. These trays were filled with fresh and clean sand (white) mixed with soil in the proportion of 1:4 ratio. The soil + sand was filled up to 5 cm depth. After filling the trays water was sprinkled to wet the sand. Twenty five treated seeds per replication were sown in rows at an equal distance in small drill. Treated and untreated seeds were put in each drill with the help of forceps and pressed gently. Later, these seeds were covered by sand. These trays were later covered with transparent polythene sheet, mounted on the upper side of the trays tightly. Within 24 h condensation of moisture appeared on the inside of the polythene sheet. As soon as germination started polythene cover was removed. Sand was kept moist by watering twice a day by sprayer. Recording was done up to 20th day regularly or till the germination continued. Germination counts were taken on the number of normal seedling, abnormal seedlings and seed rots. On the 20th day after sowing or when germination stopped the top layer of sand was sieved through a wire mesh screen and number of seeds which were either deformed or rotten were recorded.

Seedling vigour

i) Seed germination Assessment: One hundred seeds in five replications for each treatment were assessed and germination tests were conducted using petri plates with filter paper placed in them. The number of normal seedlings were counted at two days interval and cumulative germination obtained on the 8th day was recorded. Percent seed germination was then expressed as number of seeds germinated over total number of seeds plated.

ii) Seedling vigour index: Ten seedlings in each replication of germination test were collected at random from the test (final count) by paper towel method and the seedling length was measured. Seedling vigour index was calculated using the formula as method of Abdul baki and Anderson, (1973).

Seedling vigour index = Mean seedling length (cm) X Germination percentage (%).

Statistical analysis: Statistical analysis was done using Duncan's multiple range test at 5% Significance according to Snedecor and Cochan (1980).

RESULTS AND DISCUSSION

Seed infestation: Maximum fungal pathogens were

found to be associated with seeds as per the results recorded by the agar plate method and deep freeze methods. The maximum number of fugal pathogens were to be observed with the unsterilized seed samples. Farmer saved sesame seed samples from both the districts were found to be infested with ten pathogenic and five saprophytic fungi. Among the pathogenic fungi Fusarium, ALternaria, Macrophomina were the most frequent followed by curvularia spp. The less frequent pathogens were Colletotrichum and Bipolaris maydis. Aspergillus spp., Rhizopus spp and Penicillium spp were the saprophytic fungi which occurred on all the samples tested. The results showed that in all the samples, germination of sterile sesame seeds was higher than those of unsterilized seeds (Table 1). The frequency of occurrence of the pathogens in all the methods of testing was higher in unsterilized sesame seeds than in sterilized seeds. However, the unsterilized seed samples were more heavily colonized by the fungal pathogens both saprophytic and pathogenic in all the methods. These results are in agreement with those of Mbah and Akueshi (2009) who reported the presence of *Alternaria*, Helminthosporium, Fusarium, Curvularia, Rhizoctonia, Chladosporium, Aspergillus and Penicillium species in sesame seeds. On the other hand, curvularia, Fusarium, Macrophomina, Alternaria dianthicola Cecospora were reported from sesame (Kumar et al., (1984). Of all the pathoges identified, Aspergillus spp was the most abundant occurring in all the sterilized and unsterilized seeds. The consequences of such infestation is not only limited to yield losses but also accounts for the buildup of mycotoxins in infested grains. The findings of this study are therefore, important as they highlight the need for effective measures aimed at reducing seed borne infection of sesame seeds so as to take up seed production in Andhra Pradesh.

Table 1. Per cent incidence of mycoflora associated with Sessame seeds as detected by different methods.

Treatments	0	Agar Plate Method		Blotter Paper Method		2,4 D Method		Deep Freeze Method		Total	
	Steril	Unst	Ste	Unst	Ster	Unst	Ster	Unst	Ster	Unst	
Alternaria spp.	4	10	1	10	4	6	3	3	12	25	
Aspergillus spp.	5	18	2	26	5	20	4	8	16	25	
Macrophomina spp.	2	10	1	8	2	8	2	4	7	16	
<i>Cephalosporium</i> sp	2	5	1	4	1	4	1	2	5	10	
Fusarium spp.	3	8	5	12	1	8	2	6	11	10	
Curvularia spp.	3	5	2	5	2	6	2	3	9	16	
Drechsclera spp.	1	6	1	0	1	8	2	4	5	11	
Penicillium spp.	1	12	4	6	1	3	3	5	9	22	

Table 2. Effect of Bioagents and Biofertilizers on seed quality parameters of sesame seeds under laboratiory conditions (Blotter method).

Treatment	Germination (%)	Seed rot	Seedling Blight	Fungal colonies
Trichoderma + Pseudomonas fluorescence	96.0	4.50	4.18	3.83
Azatobacter + Trichoderma	94.4	8.64	6.42	10.2
Rhizobium + Trichoderma	90.2	12.1	8.63	12.6
Azatobacter	88.0	18.0	9.40	14.8
Trichoderma	85.3	10.6	7.21	12.2
Pseudomonas fluorescence	84.0	9.8	8.10	15.4
Benomil	86.3	2.70	2.10	3.00
Control	75.0	32.3	21.8	36.2
SEm±	0.48	0.72	0.80	0.94
CV%	3.71	5.46	5.68	6.78
CD	1.61	1.82	2.21	2.08

Evaluation of Seed mycoflora, Seed rot and Seedling decay: Results of study on total fungal colonies enumerated revealed that treated seeds were influenced by different treatments. Among the various seed treatments, *Trichoderma + Pseudomonas, Azatobacter + Trichoderma* were the most effective treatments in reducing seed mycoflora. Seed treatment with *Trichoderma + Pseudomonas* and *Azatobacter + Trichoderma* recorded less number of fungal colonies 3.83% and 10.2%, respectively. However the two treatments Trichoderma (12.2%), *Rhizobacter + Trichoderma* (12.6%) were not statistically significantly different and expressed in the form of total number of fungal colonies (Table 2).

Among all the treatments tested, *Trichoderma* + *Pseudomonas* (4.5%) and *Azatobacter* + *Trichoderma* (8.64%) magnified significant difference in seed rot expressions, however they were on par with seedling mortality. The results are in collaboration with Enikuomehin (2010) who reported the occurrence of *Drechsclera sorokiniana* and *Fusarium moniliforme*, *Macrophomina* as major seed borne pathogens causing seed rot and seedling blight (*Kumar et al.*1984,).

All the treatments were significantly different over control (untreated seed). Benomil reduces seed rot (2.7%) and seeding decay (2.10%) followed by *Trichoderma* + *Pseudomonas*. The toxicity of fungicide sometimes has been ascribed to affect the seed viability and the associated deterioration (Thobunluepop *et al.*, 2011). Hence the use of bio-agents might be caused to minimize the undesirable side effects and ecofriendly treatment of seed were found the best option over untreated seeds and to promote the rhizosphere colonization by beneficial fungi and to suppress the pathogenic fungi which were found to be associated in causing seed rot and seedling decay.

Effect of seed treatment on seed borne fungi: Amongst the treatments tested (Table 3), seed treatment with *Trichoderma* + *Pseudomonas*, *Azatobacter* + *Trichoderma* followed by *Azatobacter* and *Trichoderma* were found effective and reduce seed borne fungi namely *Alternaria* alternate, *A. tennissima*, *A. terreus*, *A. flavus*, *A. fumigatus* and *A. niger*, *fusarium* spp, *Macrophomina* sp and *Penicllium* spp and *Rhizoctonia solani* and *Rhizopus* stolonifer in comparison to other treatments and control. However, *Alternaria* spp (1-20%) and *Fusarium* (0-18%) by PDA method and further these are not recovered by the combination of treatments *Trichoderma* + *Pseudomonas* and *Azatobacter* + *Trichoderma*, in addition other fungi *Drechsclera* and *curvularia* were also controlled. On the other hand less per cent of *Aspergillus spp.* (3-10%), *Penicillium spp.* (2-12%), *Rhizopus spp.* (1-8%) were recovered.

Several pathogens were isolated along with the saprophytes. Minimum recovery of most of pathogenic and saprophytic fungi were recorded with all the treatments when compared to control.

Five pathogenic fungi causing seed rot and seedling blight were identified. Percent mycoflora of fusairum was abundant in control (18%) and in other treatment it was ranged from 2% (Trichoderma) and Benomyl (0%). Azatobacter + Trichoderma and Trichoderma + Pseudomonas, Macrophomina causes seedling blight and it was minimum in the Trichoderma + Pseudomonas (0%) and *Azatobacter* + *Trichoderma* (0%) and maximum incidence of mcycoflora (fusarium and Macrophomina) associated with untreated seed as 18 and 8%, respectively. Similar results of 85.2 and 73.0 % reduction in death of plants due to infection by F. oxysporium with biofertilizer in lentil and chickpea was reported Gurha et al. (2003). The antagonistic interaction of trichoderma on fusarium and macrophomina by coiling around the hyphae and penetrating into hyphae was extensively studied. (Elewa et al., 2011).

Decreased pathogenic mycoflora in treated tomato seeds with antagonistic microorganisms like trichoderma and biofertilisers combinations like *Rhizobium* + *Trichoderma* and *Azatobacter* + *Trichoderma* was earlier reported by Mogle and Mane (2010) in tomato. Similar results of reduction of seed borne pathogens of soybean by bioagents were reported (Kumar *et al.*, 1984).

Effect of treatments on germination

i) **Paper towel method:** It has been found that bioagent/biofertilizer impact on germination by paper towel method is multifold. Most of the treatments increased the germination whereas a few have retarded it when compared with that of untreated control (Table 5). Amongst the treatments combination treatments like *Trichoderma + Pseudomonas* (23%) was the most effective treatment and increased germination counts considerable over control (8%. Followed by *Azatobacter + Trichoderma* (19%), *Rhizobium + Trichoderma* (17%) when compared to control (8%).

Seed treatments with bio-agents controlled the external

	Seed rot (Preemergence mortality %)							Seedling decay (Post emergence mortality %)				
Treatments	<i>Fusarium</i> spp.	Aspergillus flavus	Aspergillus niger	Macrophomina	Alternaria	Total		Macrophomina	Fusarium sp	Alternaria sp	Aspergillus sp	Total
Trichoderma + Pseudomonas	0.4	1.7	0.8	1.6	0.6	2 4.50)	2.1	1.6	0.26	0.84	4.18
Azatobacter + Trichoderma	3.2	0.3	1.0	3.2	1.2	2 8.91	L	1.8	2.2	1.4	2.80	7.2
Rhizobium + Trichoderma	2.7	6.2	2.0	2.2	2.2	2 11.1	L	0.43	2.0	3.6	2.6	7.63
Azatobacter	3.2	2.0	2.3	3.4	4.() 15.0)	3.2	1.8	2.3	2.0	8.1
Trichoderma	3.7	2.4	2.0	1.7	3.4	ł 14.6	5	0.48	2.0	3.6	1.66	7.74
Pseudomonas	5.4	2.6	4.0	2.6	1.6	5 9.8		4.0	2.0	2.2	1.2	9.4
Benomil	1.2	0.3	0.8	1.4	4.3	3 3.7		1.6	0	1.1	0	2.7
Control	10.5	6.0	3.6	4.2	8.0) 32.3	3	10.0	8.2	3.6	10.2	21.8
SEm±						1.02	2					0.73
CV						5.46	5					3.95
CD						1.82	2					1.68
Table 4. Per cent incidence of my	coflora asso	ciated with	Sessame se	eds on Po	otato Dext	rose Agar	Mediu	ım after seed t	reatment			
Treatments	Macrophomina spp.	Aspergi-Ilus	Drechscera spp.	Cephalo spp.	Curvul spp.	Fusarium	Rhizoctonia spp.	Penicillium spp.	Chladosprium spp	Alterarua spp.	Trichoderna spp.	Rhizopus spp.
Trichoderma + Pseudomonas	0	2.0	0	0	0	0	1	2.0	2	1	8	1
Azatobacter + Trichoderma	0	2.0	0	1	1.0	0	3	2	1	2	4	2
Rhizobium + Trichoderma	1.0	7.0	0	2	2.0	4	8	6	1	3	4	4
Azatobacter	4.0	3	4.0	7	4.0	4	4	8	7	5	0	3
Trichoderma	2.1	10	1.0	6	6.0	2	7	4	7	6	10	3
Pseudomonas	4.0	8	0	4	2.0	2	6	8	5	6	0	5
Benomil	8.0	0	0	0	0	2	0	0	0	2	0	0
Control	8.0	10	12	8	4.0	18	7	12	11	20	0	10

Table 3. Effect of seed treatment with bioagents/biofertilizers on seed and seed ling decay of sesame under laboratory conditions (Blotter method).

and internal seed borne pathogens and there by acting as a protective coating to prevent soil borne pathogens from seedling diseases. Similar observation were reported workers in sesame (Elewa *et al.*, 2011 and El-Sayed *et al.*, 2011). Benomil was found effective in reducing the frequency of total fungal recovery Table. with reduced seed germination percentage.

ii) **Sand Method:** In this method the treatment combinations substantially increased the germination percentage as compared to untreated control. Whereas treatments Benomil and *Pseudomonas* and *Azatobacter* were neither increased nor decreased germination and germination counts were similar in *Azatobacter* + *Trichoderma* and *Rhizobium* + *Trichoderma* treatment combinations.

iii) Effect of treatments on seedling vigour: All the seed treatments involving the bioagents /biofertilizers

recorded high seedling vigor ranging from (904 to 1660) over untreated control (Table 5). The treatment combination fully exhibited seed vigour index than other treatments. These results are in agreement with the findings of improved seedling vigor with organic seed treatments over inorganic treatments (Oyekale *et al.*, 2012).

From the study it infers that presence of pathogen on seed surface decreased by mixed inoculation of bioagent and biofertilizer in turn it may increase the rate of germination so that weakened seeds may survive to produce normal plantlets. It can be concluded that seed dressing with mixture of bioagents and biofertilizers may be beneficial in reducing the intensity of seed borne diseases and enhancing seed germination percentage of sesame seeds.

Table 5. Effect of bioagents/biofertilizers seed trat on per cent germination of sesame seeds as determined by paper towel method.*

Treatments	Paper Towel Method				Sand Method				Seedling Vigor
	NS	AS	SR	HS	NS	AS	SR	HS	
Trichoderma + P. fluorescence	23	0	14	63	10	2	0	88	1660.3
Azatobacter + Trichoderma	19	2	18	61	5	4	0	92	1562.6
Rhizobium + Trichoderma	17	3	26	52	5	2	4	90	1404
Azatobacter	14	6	32	48	1	3	0	94	1386.2
Trichoderma	12	2	30	50	3	2	2	93	1489
Pseudomonas fluorescence	8	4	16	54	1	4	2	93	1356.3
Benomil	10	2	2	72	2	2	0	96	1312
Control	8	3	47	42	1	1	8	91	904.1
SEm±									69.2
CV									245.6
CD									121.2

*Viability = 76 per cent, ** Data on germination is based on 100 seeds, *** Data based on observation of normal seedling. NS = Normal seedling, AS= Abnormal Seedlings. SR = Seed rots, HS = Hard seeds.

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