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COMPATIBLE ROLE OF SEA WEED EXTRACTS AND CARBENDAZIM ON THE BIOLOGICAL ACTIVITIES OF PADDY

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ABSTRACT

Seaweed extracts which enhanced the seedling vigour; seed quality can also be used along with Carbendazim for the better performance to get productive plants in field which was indicated by better germination and growth. In parallel, it showed increased activity of amylase, proved its necessity as energizer for increased seed germination and growth with a great expectancy in more yield in Dravya and Green plus combination. Sea weed extracts (SWE) also proved better through the reduced incidence of seed mycoflora in combination with Dravya, Green plus and Cabendazim.

KEYWORDS: Sea Weed Extracts, Paddy, Seed Mycoflora, Seedling Germination, Seedling Vigor, Carbendazim

INTRODUCTION

Paddy (*Oryza sativa* L.) is one of the important staple food crop, which is being cultivated in the large area in India for its nutritive value. India has the largest paddy output in the world and is also the fourth largest exporter of rice in the world. Paddy is cultivated at least twice a year in most parts of India, the two seasons being known as Rabi and Kharif, respectively. Out of the total production, 90% of paddy is being consumed in Asian countries (Ali, 2004). The paddy cultivation plays a major role in socio-cultural life of rural India. Commonly, cultivars like IR-64, Tanu, Raksha, Jaya, Mandya vijaya are being cultivated in southern part of India. Among these, IR-64 comparatively a short duration, high yielding variety has got high demand for marketing in South Karnataka. So, when such a worthy crop is known to affect by various diseases, such as blight, blast, leaf spots, rot, scald of foliage's including several panicle diseases due to fungal and bacterial pathogen, it is necessary to manage at every steps. In order to manage these, variety of fungicides and bactericides are being used along with seeds as well as in the fields. The growing population is facing pressure on food production and to meet the increasing demand, farmers are using chemical fertilizers to enhance their crop production. Chemical fertilizers mixed with pesticides get accumulated in plants which lead to health problems in human and domesticated animals due to bio-magnification and in total cause hazardous effect to the environment (Hansra, 1993). So, it is necessary to safe guard it through eco-friendly manner using bio-based products. After the evaluation several aspects of seed germination and seedling growth problems in paddy and in order to overcome or to improve problems, in the present study emphasis has been made **t**o evaluate the efficacy of seed treatment with sea weed extracts and carbendazim in the enhancement of seed germination and growth promotion. Apart from these, the effect of Sea weed extracts (SWE) and carbendazim was also assessed with respect to amylase activity and seed borne fungi of paddy.

MATERIALS AND METHODS

Seed samples of paddy cultivar IR-64 were collected from Karnataka State Seed Corporation (KSSC) Bangalore,

Karnataka state; India and were stored in polythene bags at 10ºC for further use. Each seed sample was mixed thoroughly in a seed sampler and a working sample was drawn and used for germination studies. Among the seed sample of cultivar IR-64, a sample having low germination percentage was selected for the entire study.

Brown algal sea weed extract products like Dravya, Green plus and Phyton-T were collected from Green Life Technologies, Pvt. Ltd., Mysore and were used separately at their respective recommended dosage of 1ml/1t of Dravya, 1ml/5lt of Green plus, 5ml/lt of Phyton-T for seed treatment 0.5g/lt of Carbendazim was used individually and along with SWE. The SWE were also used for seed treatment in combinations of 50% recommended dose each and also in combination with carbendazim (50% of 0.5g/lt).

Evaluation of Sea Weeds Extract and Carbendazim for Their Effect on Seed Germination and Seedling Vigor in Paddy

In the present study, the seeds were subjected to soaking for 16h at 28±2°C in the solution of 1ml/lt of Dravya, 1ml/5lt of Green plus, 5ml/lt of Phyton-T, with 0.5g/lt, Carbendazim alone and in combinations. Whereas the seeds soaked in water served as control. Soaked seeds of each combination were air dried; 100 seeds of 4 replicates of each treatment were plated equidistantly on two layers of wet blotter towels (34×14.5 cm) and were covered with another wet blotter sheet, then rolled and incubated for a period of 14 days at 22±2ºC under 12/12h of alternate cycles of light and darkness, according to the procedures of ISTA (Anonymous, 1996). On 14^{th} day of incubation, the seedlings were examined carefully for essential structures, the normal seedlings were counted, the percentage seed germination, root and shoot length were recorded. Finally, their vigour index was calculated.

Assessment of Amylase Activity in Sea Weed Extract Treated Paddy Seedlings

On the other hand, based on the promising seed treatment, seedlings were subjected to the amylase assay. For this purpose, seeds of effective treatment were sown in wet sand filled in plastic trays. One to five days old seedlings were harvested, washed and used for enzyme extraction. Similarly, seedlings raised out of control (seeds treated with water), also used for enzyme extractions for comparison.

Amylase activity in the seedlings was determined following the procedures as described by Jayaraman (1981). Starch is the major component of most of the world's crop yield and the degradation of starch is essential in the seed germination of the plants species (Yoshiki and Yamasaki, 2003). Starch degradation in seeds requires the action of beta-amylase and alpha-amylase. Hence, in the present study amylase activity has been considered as an important factor with respect to varied seed treatment. For this purpose, 2g of paddy seedlings (1,2,3,4 and 5^{th} day old seedling at separate set) of different treatment were harvested and ground into paste in a pestle and mortar by adding 2ml of ice cold 0.1M phosphate buffer solution of $_{P}$H 6.7. The temperature was maintained at 4ºC by putting ice in the outer chamber of the pestle in mortar (homogenizer). The suspension was then filtered through 3 layers of muslin cloth in cold condition. The filtrate was collected and clarified further by centrifugation in a refrigerated centrifuge at 10,000 rpm for 15 minutes at 4ºC, in which 1% starch solution was used as substrate. The amylase activity was measured by estimating the release of maltose calculated from the standard curve prepared with maltose. One unit of amylase activity was defined as the amount required for liberating 1 mg of maltose in 15 min at 37º C.

Evaluation of Sea Weed Extracts and Carbendazim for Their Effect on Seed My Coflora of Paddy

Safeguarding the seeds from fungal attack is the main task in agriculture. So, in order to manage, seed-borne

infections, sea weed extracts, which were thought to be eco-friendly, were used along with Carbendazim. Accordingly, seeds were subjected to soaking for 16h at 28±2ºC in the solutions of Dravya-1ml/lt, Green plus-1ml/5lts and Phyton-T-5ml/lt with the addition of 0.5g of Carbendazim and also in combinations with SWE, where seeds soaked in water served as corresponding control. Such treated seeds were further blotted and air dried for 24hours. 400 seeds of each set of treated seeds were placed on wet blotter discs taken in the plastic plates of 9cm diameter and incubated for a period of 7days at 22±2ºC 12/12hours alternate cycles of near UV light and darkness. On 7th day, the plates were evaluated under the stereo binocular and compound microscopes. The per cent incidence of fungi was recorded and the data were tabulated.

RESULTS AND DISCUSSIONS

In the present study, data recorded revealed the stimulatory effect of the individual seed treatment pertaining to enhanced seed germination and seedling vigour. In all the treatment seedlings were found apparently healthy with lengthy roots compared to control (Table 1).

Table 1: Stimulatory Effect of SWE on Seed Germination, Root-Shoot Length and Vigour Index of Paddy (IR-64)* Enhanced Activity of Seed Treatment with SWE

Seed Treatment With SWE	%Seed Germination	MRL±SE	MSL±SE	Vigour Index (VI)
Control	83	4.63±0.01	4.92±0.02	792.65
Dravya (D)	89	7.92±0.03	8.46±0.02	1457.82
Green plus (G)	86	7.44±0.03	7.62±0.02	1367.40
Phyton-T (P)	85	7.21±0.02	7.53±0.04	1252.9

*Data based on average of 400 seeds/ replicate of 100 seeds each.

SWE= Sea weed extract, MRL± SE = Mean root length ± standard error, MSL± SE = Mean shoot length ± standard error,

In case of root and shoot length, the data were recorded on the average of 25 seedlings from each replicate. In each case vigour was calculated based on the following formula;

(MRL+MSL) ×% of seed germination

Among sea weed extracts which are known to be eco-friendly, Dravya has increased 6% seed germination, mean of root-shoot length was found to be enhanced over control 7.92, 8.46cm, respectively. The vigour index was increased two fold over control. In Green plus, Phyton-T, it was enhanced by 3%, 2%, respectively with increase in root, shoot length with enhanced vigour respectively. Compared to the control, where 83% in germination, 4.63, 4.92cm in mean root, shoot length and 792.65 of vigour index were recorded.

Sea weed extracts showed the better performance in the recovery of abnormal seedlings into normal seedlings to some extent (Table 2).

Table 2: Effect of SWE on Abnormal and Dead Seedlings of Paddy (IR-64) as Observed in Paper Towel Method % Seedlings with Deformity

Seed Treatment With SWE	% of ABN	% of DS	% of Seed Improvement out of Abnormal Seedlings
Control	7	10	0
Dravya (D)	2	9	29
Green plus (G)	5	9	71
Phyton-T (P)	5	10	71

C=Control, D=Dravya, G=Green plus and P=Phyton-T

Control, where it showed 7% abnormal seedlings, 10% dead seedlings comparatively, Dravya, Green plus and Phyton-T treatment showed 2, 5, 5% abnormal seedlings and 9, 9, 10% dead seedlings, respectively. Among the SWE Dravya showed better effect and found best in strengthening the weak seedlings to grow as normal seedlings, which will grow as future productive plants. Data recorded in tabled 3 revealed the efficacy and compatibility of sea weed extracts with extracts with Carbendazim in enhanced growth of seedlings (Table 3).

Table 3: Compatibility of Sea Weed Extracts with Carbendazim For Enhanced Seed Germination and Seedlings Growth of Paddy (IR-64)

SWE in Combination with Carbendazim	% Seed Germination	MRL±SE (Cm)	MSL±SE (Cm)	Vigour Index (VI)
C	83	4.63±0.01	4.92±0.02	792.65
D+Cr	88	7.80±0.10	8.02±0.02	1392.16
G+Cr	86	7.48±0.08	7.64±0.11	1300.32
P+Cr	86	7.28±.07	7.74±0.10	1291.72
D+G	90	8.2±0.17	8.6±0.12	1512.00
D+G+Cr	89	8.1±0.09	8.3±0.16	1459.60
D+P	84	7.38±0.02	7.86±0.03	1280.16
D+P+Cr	85	7.42±0.04	7.9±0.04	1302.20
D+G+P	87	7.46±0.5	7.92±0.06	1338.06
D+G+P+Cr	87	7.42±0.06	7.98±0.10	1339.80
G+P	85	7.10±0.10	7.42±0.02	1234.2
G+P+Cr	86	7.21±0.03	7.48±0.05	1263.34

SWE=Sea weed extract, MRL=Mean root length in cm, MSL=Mean shoot length in cm, SE=Standard error, C=Control, D=Dravya, G=Green plus, P=Phyton-T, Cr=Carbendazim.

Along with Carbendazim, seed germination % was found to increase by 5% in Dravya, 3% in Green plus, 3% in Phyton-T, correspondingly, enhanced root-shoot length and vigour index. This attempt has proved enhanced seed germination %, vigour index, under reduced concentration by 50% of its original recommended concentration of SWE along with Carbendazim.

Combinations of Dravya and Green plus were found better, where it showed 7% increase in germination, whose root- shoot length were enhanced to 8.2 and 8.6cm, in which vigour index was found to be 1542.00. Compared to control, Carbendazim along with the SWE showed better results, which was found to be 6% in D+G+C, 1% in D+P, 2% in D+P+C, 4% in D+G+P and D+G+P+C, 2% in G+P, 3% in G+P+C with respect to improvement in root-shoot length and vigour of the seedlings.

Table 4: Effect of Sea Weed Extracts in Combination With Carbendazim on the Recovery of Abnormal Seedlings of Paddy (IR-64)* % Improved Seedlings Due to SWE Treatment

SWE in Combination with Carbendazim	% of ABN	% of DS	% ABN Seedlings Transformed to Normal
C	7	10	0
D+Cr	3	9	43
G+Cr	5	9	71
P+Cr	4	10	57
D+G	2	8	29
D+G+Cr	2	9	29
D+P	7	9	100
D+P+Cr	6	9	86
D+G+P	5	8	71
D+G+P+Cr	5	8	71
G+P	5	10	71
G+P+Cr	4	10	57

*Data based on average of 400 seeds/ replicate of 100 seeds each.

C=Control, Cr=Carbendazim, D=Dravya, G=Green plus, P=Phyton-T, ABN=Abnormal seeds, N=Normal seeds, DS=Dead seeds

Treatment in combination with SWE and Carbendazim showed better performance in the recovery of the abnormal seedlings into healthy seedlings (Table 4). In combination with Carbendazim, Dravya, Green plus and Phyton-T treated seeds showed 3, 5, 4 abnormal seedlings and 9,9,10 dead seedlings, respectively, Compared to other treatment in Dravya and Green plus combination treated seeds showed 2% abnormal seedlings and 8% dead seeds, even compared to their respective control.

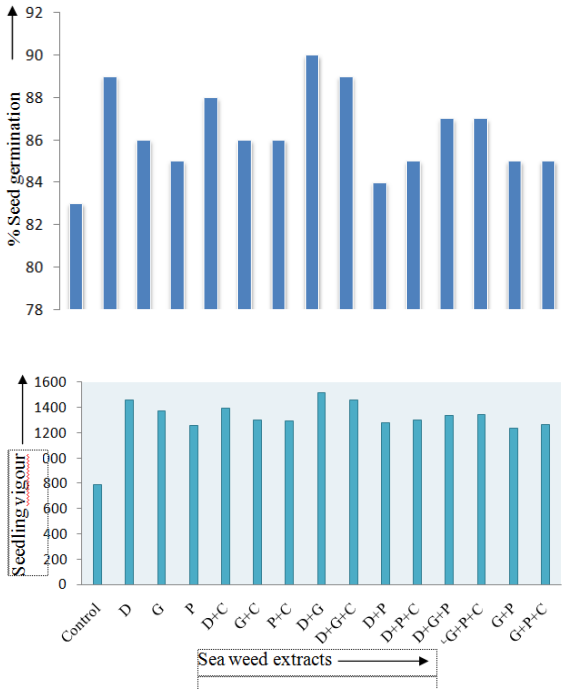

Figure 1: Paddy Seed Treatment with SWE in Combination with Carbendazim Indicated the Enhanced Growth of Seedlings

C=Control, D=Dravya, G=Green plus, P=Phyton-T, Cr=Carbendazim

The data in the figure 1 indicated the better performance of different combination of SWE with Carbendazim. Accordingly, D+G was found effective in enhancing the seedling growth and % of seed germination over any other treatment performed.

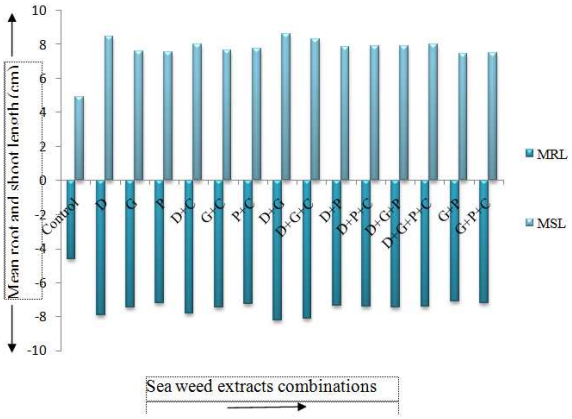

Figure 2: Compatibility of SWE with Carbendazim in Enhanced Growth of Root and Shoot of Paddy

C=Control, D=Dravya, G=Green plus, P=Phyton-T, Cr=Carbendazim, MRL=Mean root length, MSL=Mean shoot length

Dravya and green plus combination improved the root-shoot length comparatively, followed by their Dravya alone remained potential over control, indicated apparently healthy and vigorous seedlings.

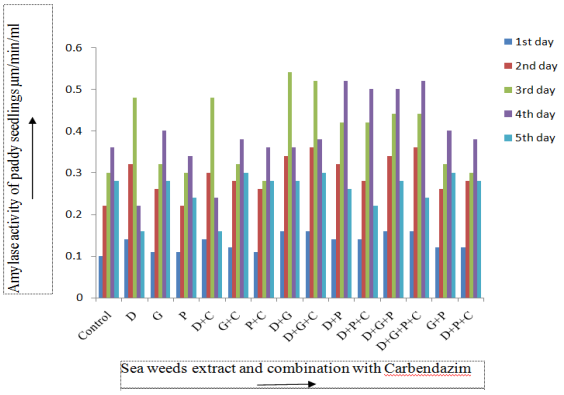

Figure 3: Combined Effect of SWE and Carbendazim on the Amylase Activity in Paddy Seedlings

Based on the efficacy, the treated seedlings were subjected to the enzyme assay. From day1, enzyme activity gets started as represented in figure 4, which elaborates the details of the amylase activity in treated seedlings. Compared to the control, all treated seedlings showed increased amylase activity. The data revealed that in control there was little enzyme activity 0.87µm of maltose/min/ml of enzyme at room temperature in day-4. Dravya, Green plus and Phyton-T treated seedlings showed 1.16, 0.96 and 0.93µm/min/ml of enzyme activity at day 3, 4 and 5, respectively. In combination of Dravya, Green plus and Phyton-T with Carbendazim showed 1.16µ, 0.93 and 0.87µm/min/ml of enzyme activity at day 3, 4 and 5, respectively Finally, it was found that in combination of SWE with Carbendazim, there was greater activity in Dravya and Green plus combination in day 3 which was 1.30µm/min/ml activity must have been played a role in enhanced seed germination and seedling development, where as in combination of Dravya+Green plus+Carbendazim, Dravya+Phyton-T along with Carbendazim, Dravya+Green plus+ Phyton-T along with Carbendazim and Green plus+

Phyton-T along with Carbendazim 1.25, 1.25, 1.25, 1.21, 1.25µ, 0.96 and 0.93 µm of maltose/min/ml of enzyme at room temperature, was noticed at day-3 and day-4, respectively.

Table 5: Synergistic Effect of SWE and Carbendazim on Seed Mycoflora of Paddy (IR-64)* % Incidence of Fungi in Seeds

Seed Treatment	Alternaria Alternata	Bipolaris Oryzae	Curvularia Lunata	Fusarium Moniliforme	Fusarium Solani
Control	18	36	4	12	14
Dravya	14	24	3	9	12
Green plus	15	30	4	10	12
Phyton-T	17	32	4	10	10
D+C	12	20	2	5	6
G+C	14	25	3	8	10
P+C	15	26	2	8	8
D+G	10	16	-	6	7
D+G+C	6	12	-	6	6
D+P	12	22	2	8	6
D+P+C	10	20	2	6	6
D+G+P	9	18	1	5	7
D+G+P+C	8	18	2	5	7
G+P	9	15	2	5	8
G+P+C	8	15	-	4	2

*Data based on average of 400 seeds/ replicate of 100 seeds each.

C=Control, D=Dravya, G=Green plus, P=Phyton-T, Cr=Carbendazim

Seaweed extracts are also having antifungal properties, which along with Carbendazim showed better results in suppressing the fungal activity on seeds. As been comparing to the control Dravya, Green plus and Phyton-T treated seeds; it showed the reduced % of incidence of *Alternaria alternata, Bipolaris oryzae, Curvularia lunata*, *Fusarium moniliforme* and *Fusarium solani*. In combination with Carbendazim and Dravya showed 1 fold reduction in the incidence of *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium moniliforme* and *Fusarium solani*. Dravya and Green plus along with Carbendazim showed complete reduction of *Curvularia lunata*, 2 fold reduction in the incidence of *Alternaria alternata* and *Bipolaris oryzae*, 1 fold reduction of the incidence of *Fusarium moniliforme* and *Fusarium solani*. Finally, Green plus, Phyton-T and Carbendazim showed complete reduction of *Curvularia lunata*, 6, 3, 3 and 1 fold reduction in *Fusarium solani, Fusarium moniliforme*, *Bipolaris oryzae* and *Alternaria alternata*, respectively. In this study, it was observed that there was reduction of brown spot disease causing pathogen *Bipolaris oryzae* due to Dravya, Green plus and in combination with Carbendazim than alone.

Seed samples shows less germination, in spite of special care taken during storage. As the result, the farmers experience great loss. So, that they use many pesticide against pest and diseases. The present study, proved the effectiveness of seed treatment on seed germination, seed vigour and seed health through eco-friendly sea weed extract, which contains macro, micro nutrients serves as phytotonic. Seed industries are often facing severe loss due to loss of entire lot which is at the margin of meeting germination. By treating with this SWE it is possible to overcome these problems facilitate the seed producers and traders it save their product which are at the range of economic danger.

In the present investigation, low concentration of SWE which are diluted in water, gave better results pertaining to enhanced germination. The control, which showed 83% germination and vigour index, was found to be 792.65, but in combination treatment with Dravya and Green plus which was 7% in germination and vigour index was1512. But, Salvem

and Shivakumar (2013) found that, compared to control i.e. 78.05% in germination and in low concentration treatment it improves up to 100% in germination. These findings are comparable with the observations of the present study.

Many crops seeds, suffer from biotic stress, which initiates from field, persist and elaborate in storage due to pathogens attack, leads to loss of germination and ultimately reduce the yield. In order to overcome these constraints, SWE proved promising for the agriculture industries as well as for the farmers. *Bipolaris oryzae* which cause brown spot, *Curvularia lunata* and *Fusarium moniliforme* cause pecky rice (Kernel spot) disease in paddy, respectively. Hence, for the beneficiaries would be potent in reduce their percent occurrence to a greater extent and definitely play a significant role comparatively in safeguarding the suffering seed lot either by seed industries or by any growers.

Finally, the treated seeds are also known to involve in the induction of defense mechanism to overcome the pathological constraints results in the improved seed germination. Rahman *et al*. (2007) are of the similar opinion in which enhanced amylase activity and correlated the same with the enhanced seed germination.

The phyto-stimulatory activity of SWE might also be due to their triggering activity in triggered synthesis of phyto-hormones or otherwise they themselves might have acted as growth promoting factors. On the other hand, it is also probably due to enhanced synthesis of phenolics in the host tissues, during the early growth of seedlings which might have arrested the proliferation of biotic factors. These combinations also might have performed in suppressing the bothering microbes either fungi or bacteria and thus might have facilitated seed to geminate. It is also possible to claim that these treatments might have antimicrobial properties, played an important role in safe guarding the seedlings. Other possibilities are also perhaps due to their involvement as energizer during early emergence of root-shoot in which they render energy by enhancing enzyme activity by their involvement in the other biochemical pathways during germination.

In present study, effort have been made using bio based product i.e. SWE, to improve the seed quality in view of increasing the yield, which are not hazardous to environment and comparatively economical. Dravya and Green plus proved better in enhancing seed germination and seedling vigour. In parallel, it showed more increased activity of amylase, proved their necessity as energizer for increased seed germination and it also involved in suppressing seed-borne mycoflora due to Green plus, Phyton-T and Carbendazim in combinations rather than individual treatment.

Seaweed extracts which enhanced the seedling vigour, can also be used along with Carbendazim for the better performance to get productive plants in field. Apart from the enhanced seed germination, SWE also played an important work in the reduced incidence of seed mycoflora, hence will perform as a promising treatment for the growers. It gives an idea for its usage as subsequent treatment as foliar spray at different schedules in the field. Since, these are eco-friendly can be made use of with further combination with other phytotonics as well as bio-organics like plant extracts vermicompost, leahates for the betterment of sustainable agriculture. The formulations of their combinations may also favour the growers to uplift the economy and also serve better in reducing the incidence of seed-borne infection effectively in the field.

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