

INDIVIDUAL AND COMBINED EFFECTS OF KRILAXYL AND GAUCHO ON CHLOROPLAST PIGMENTS, RELATIVE WATER CONTENT AND EARLY GROWTH ATTRIBUTES IN RICE SEEDLINGS, DURING GERMINATION UNDER WET TREATMENT

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The present research work has been conducted to find out the effects of individual and incombination seed treatments by Krilaxyl (*a fungicide*) and Gaucho (*an insecticide*) on Rice (*Oryza sativa* L.) cv. Mangala under different exposure periods of 12, 24, 48 and 96 hrs respectively. Wet treatment of both the pesticides had exhibited negative response on germination, early seedling growth, fresh weight and dry weight at recommended, increased and in combination treatments of all the exposure periods. Lower concentrations of lower exposure periods did not affect much on germination but, higher doses of longer exposure periods delayed the germination of seeds. There is a drastic change in chloroplast pigments (*Chl. 'a', 'b', total and Carotenoids*) and the percent relative water content (*both in leaves and seedlings*) have been decreased along with the increased concentrations by both the pesticides over control. Calculated results of vigour index and tolerance index have also been decreased and the level of toxicity have been increased at higher and incombination dosages of all the exposure periods. At longer exposure periods of all the concentrations, the ratio of root and shoot decreases and the ratio of chlorophyll 'a' and 'b' increased significantly as compared to the control.

Keywords : Chloroplast pigments; Dry Weight; Early growth; Fresh Weight; Gaucho; Germination; Krilaxyl; Relative Water Content.

Introduction

Rice (*Oryza sativa* L.) is the most important crop in the world today. It is the major dietary staple food for a large percentage of the world's population particularly in Asia. One of the major cause for the loss of food productivity in terms of quantity and quality is due to incidence of many pests. For the control of these early pests, several of the insecticides and fungicides applied to the seeds have been observed to produce profound biological effects on seedlings¹⁻³. Most of the pesticides are not highly selective. Unfortunately, among several toxic chemicals, few are even unknown or unidentified to the biota and high level of pesticidal residues in food items are also of great concern⁴.

Besides, other methods of pesticidal application, seed treatments, seed dressings have also shown that, they react with cellular constituents producing both germination and physiological changes during early seedling growth^{5,6}. Present attempt has been undertaken to study, the individual and combined effects of fungicide (Krilaxyl) and insecticide (Gaucho) on chloroplast pigments, relative water content and early seedling growth of Rice (*Oryza sativa* L.) cv. Mangala, during germination under wet treatment.

Material and Methods

The seeds of Rice (*Oryza sativa* L.) var. Mangala was

procured from a V.C. farm, Mandya. Krilaxyl (*a fungicide*) and Gaucho (*an insecticide*) were obtained from authorised dealers, Bangalore. The seeds were separated for uniform size, shape and color, which were used for germination experiments. Prior, to treatment the seeds were surface sterilized with 0.1% HgCl₂ for two minutes. 25 gms of seeds were taken for each treatment and the seeds were thoroughly mixed with prepared chemical slurry of different dosages viz., recommended dosages (Krilaxyl - 7gms/kg and Gaucho - 10gms/kg of seeds), 25% of the above recommended, 25% of the below recommended and in combination of both the pesticides, 50% among recommended dosages and kept for different exposure periods of 12, 24, 48 and 96 hrs respectively. Then, the seeds were subjected for germination using paper towel method. The seeds were germinated in distilled water as control and replicated for 4 times.

On termination day (ISTA⁷) of the experiment, the percent germination, length of radicle, plumule, fresh weight and dry weight were recorded. Chlorophyll⁸ and Carotenoides⁹ and Relative water content¹⁰ of both leaves and seedlings were estimated. Vigour index, Tolerance index and Percent phytotoxicity were calculated by using the standard formula prescribed by Abdul Baki and

Table 1.

CROP		<i>Oryza sativa</i> L. cv. Mangala.									
TREATMENT	EP	GERM (%) AM±SD	MRL (cms) AM±SD	MSL (cms) AM±SD	R/S AM±SD	VI AM±SD (%)	T.I. AM±SD	P.P (%) AM±SD (%)	Fresh wt. (gms) AM±SD		
BR	12H	84.00±1.04	15.6610±0.56	18.9612±0.56	0.8259±0.02	2908.26±89.36	87.72±2.60	12.27±0.29	4.14±0.02		
	24H	78.61±2.32	13.8392±0.41	18.2991±3.60	0.9341±0.10	2265.79±13.56	84.16±1.62	16.16±1.65	2.37±0.00		
	48H	68.80±2.45	8.2670±0.09	9.3748±0.30	0.8829±0.03	1213.13±27.27	71.72±3.74	28.29±3.74	1.54±0.01		
	96H	60.30±2.28	6.7714±1.14	8.4414±0.39	0.8021±0.06	917.33±26.41	67.16±0.89	32.83±3.45	0.91±0.02		
RD	12H	69.41±1.16	12.0641±0.27	13.4565±0.03	0.8965±0.01	1771.38±32.20	67.75±0.66	32.41±2.64	2.42±0.15		
	24H	63.59±2.15	9.1747±0.30	11.7659±0.51	0.7805±0.02	1332.68±85.4	55.81±2.73	44.17±2.13	1.55±0.08		
	48H	56.11±3.94	6.7445±0.17	7.8311±0.35	0.8580±0.01	728.71±40.05	58.45±1.89	46.53±1.89	0.85±0.03		
	96H	51.00±0.88	5.1014±0.08	6.2330±0.02	0.8184±0.02	578.05±25.20	50.60±0.09	49.39±0.57	0.66±0.05		
AR	12H	59.64±2.82	7.9660±0.28	8.6014±0.35	0.9261±0.03	988.41±23.41	44.62±0.88	55.37±2.02	1.72±0.24		
	24H	59.59±2.30	6.6405±0.36	9.1485±0.40	0.7258±0.02	847.67±60.93	40.41±2.63	59.57±2.57	1.02±0.02		
	48H	45.88±2.28	5.8287±0.12	7.0057±0.29	0.8329±0.02	562.37±14.50	50.58±2.92	49.41±2.92	0.62±0.05		
	96H	40.30±1.51	5.0010±0.08	6.1211±0.14	0.8170±0.01	448.22±12.01	49.12±1.50	50.39±1.62	0.49±0.03		
BR	12H	82.00±1.08	13.4410±0.36	15.2014±1.02	0.8841±0.02	2348.70±67.20	75.29±3.56	24.70±3.04	3.62±0.60		
	24H	68.66±2.51	10.2266±0.39	10.9051±0.15	0.9374±0.02	1449.86±87.20	62.12±2.52	37.78±3.04	1.35±0.07		
	48H	58.33±2.35	6.4238±0.11	7.6716±0.33	0.8382±0.02	823.00±57.20	44.24±3.21	44.24±3.26	1.43±0.02		
	96H	52.20±0.68	5.1024±0.60	6.7162±0.19	0.7596±0.08	616.95±11.36	50.61±1.54	49.38±1.50	0.88±0.03		
RD	12H	70.44±0.73	10.1120±0.26	11.3603±0.38	0.8901±0.03	1512.50±37.82	56.64±2.82	43.35±0.62	2.18±0.03		
	24H	57.07±2.07	8.1264±0.43	9.3010±0.94	0.8782±0.04	992.99±62.60	49.44±2.32	50.54±2.95	1.90±0.04		
	48H	38.44±2.43	5.3397±0.23	6.2291±0.29	0.8573±0.00	445.19±40.46	46.38±3.96	53.60±3.96	1.27±0.93		
	96H	32.30±0.44	4.6100±0.11	5.4040±0.83	0.8530±0.02	323.45±11.25	45.72±0.88	54.27±2.03	0.70±0.02		
AR	12H	57.00±2.82	8.7166±0.36	9.3331±0.24	0.9339±0.10	1028.83±36.15	48.82±1.50	51.17±2.06	1.32±0.36		
	24H	45.45±0.14	6.2380±0.56	7.4409±0.88	0.8411±0.02	672.98±133.4	37.99±3.93	62.10±3.93	0.73±0.02		
	48H	31.77±2.26	4.4648±0.29	5.3145±0.31	0.8404±0.03	311.01±31.27	38.75±3.38	61.23±3.43	0.44±0.01		
	96H	28.66±1.96	4.0124±0.20	4.7160±0.05	0.8508±0.01	250.15±9.46	31.80±1.05	63.19±2.62	0.32±0.01		
Gaicho + Krilaxyl	12H	48.70±1.64	7.6130±0.43	8.2322±0.31	0.9247±0.02	771.66±4.31	42.64±2.82	57.35±0.09	1.21±0.11		
	24H	41.82±1.76	6.0888±0.38	6.8944±0.25	0.8863±0.08	542.94±21.17	37.05±1.92	62.98±1.93	1.12±0.02		
	48H	33.51±2.48	4.1081±0.08	5.2802±0.34	0.7808±0.04	323.91±17.51	35.62±1.52	64.36±1.52	0.32±0.00		
	96H	23.90±2.17	2.7661±0.06	3.2462±0.34	0.8521±0.01	143.69±9.76	27.43±0.72	72.56±0.72	0.19±0.04		
CONTROL	12H	99.66±2.54	17.8514±0.46	19.0101±0.40	0.9390±0.03	3673.61±108.91	---	---	5.84±0.10		
	24H	90.22±4.35	16.4408±0.24	18.6292±0.48	0.8830±0.02	3161.88±110.04	---	---	3.55±0.08		
	48H	78.66±2.12	11.5490±0.46	13.7299±0.42	1.0795±0.08	1988.43±55.95	---	---	1.80±0.04		
	96H	73.70±1.18	10.0810±0.12	11.0021±0.38	0.9162±0.01	1553.82±37.82	---	---	1.06±0.05		

Germination percentage based on normal seedlings only.

GERM : Germination. MRL : Mean Root Length, MSL : Mean Shoot Length, R/S : Root & Shoot Ratio, VI : Vigour Index, T. I : Tolerance Index, P.P : Percentage Phytotoxicity, EP : Exposure Period, H : Hour, BR : Below Recommended, RD : Recommended Dosage, AR : Above Recommended, I+F : Insecticide + Fungicide, WT : Weight, AM : Arithmetic Mean, SD : Standard Deviation.
Value are represented as Arithmetic mean and Standard Deviation of four determinants.

Table 2.

CROP		Oryza sativa L. cv. Mangala.									
TREATMENT	EP	CHL'a' (mg.g ⁻¹) AM±SD	CHL'b' (mg.g ⁻¹) AM±SD	TOT CHL (mg.g ⁻¹) AM±SD	CHL.a/b Ratio AM±SD	CAR (mg.g ⁻¹) AM±SD (%)	RWC in Seedlings AM±SD (%)	RWC in Leaves AM±SD (%)	Dry wt. (gms) AM±SD		
BR	12H	0.0754±0.00	0.0604±0.00	0.1349±0.00	1.2334±0.93	0.0614±0.00	84.09±1.62	93.72±4.35	0.74±0.01		
	24H	0.0644±0.00	0.0586±0.00	0.1230±0.00	1.0993±0.03	0.0459±0.00	82.61±0.47	90.03±0.74	0.46±0.00		
	48H	0.0535±0.00	0.0508±0.00	0.1045±0.00	1.0518±0.00	0.0401±0.00	72.58±0.10	86.66±0.29	0.32±0.00		
	96H	0.0485±0.00	0.0440±0.00	0.0925±0.00	1.1022±0.02	0.0476±0.00	63.60±3.93	79.31±2.32	0.27±0.00		
RD	12H	0.0585±0.00	0.0525±0.00	0.1110±0.00	1.1142±0.93	0.0395±0.00	78.62±3.74	88.93±0.73	0.62±0.00		
	24H	0.0417±0.00	0.0426±0.00	0.0844±0.00	0.9799±0.04	0.0286±0.00	70.38±0.39	86.80±0.24	0.37±0.00		
	48H	0.0407±0.00	0.0372±0.00	0.0546±0.00	1.0962±0.02	0.0310±0.00	64.73±0.12	80.37±0.83	0.27±0.00		
	96H	0.0378±0.00	0.0302±0.00	0.0680±0.00	1.2516±0.11	0.0354±0.00	56.20±2.73	73.00±2.06	0.20±0.00		
AR	12H	0.0412±0.00	0.0464±0.00	0.0888±0.00	0.9137±0.03	0.0235±0.00	75.11±6.73	77.61±1.08	0.46±0.02		
	24H	0.0281±0.00	0.0293±0.00	0.0570±0.00	0.9625±0.07	0.0192±0.00	73.17±0.20	75.35±0.25	0.22±0.00		
	48H	0.0213±0.00	0.0232±0.00	0.0442±0.00	0.9210±0.03	0.0216±0.00	60.74±0.42	74.08±0.25	0.16±0.00		
	96H	0.0202±0.00	0.0246±0.00	0.0448±0.00	0.8211±0.02	0.0282±0.00	51.44±2.55	68.30±1.52	0.12±0.01		
BR	12H	0.0722±0.00	0.0576±0.00	0.1298±0.00	1.2534±0.02	0.0565±0.00	78.06±3.94	91.33±4.72	0.52±0.01		
	24H	0.0693±0.00	0.0478±0.00	0.1207±0.00	1.4656±0.13	0.0530±0.00	75.24±0.75	85.64±0.49	0.35±0.00		
	48H	0.0553±0.00	0.0408±0.00	0.0962±0.00	1.3567±0.02	0.0414±0.00	67.15±0.32	82.24±0.22	0.29±0.00		
	96H	0.0444±0.00	0.0392±0.00	0.0836±0.00	1.1326±0.05	0.0386±0.00	58.32±2.82	71.08±6.26	0.22±0.01		
RD	12H	0.0636±0.00	0.0455±0.00	0.1091±0.00	1.3978±0.07	0.0402±0.00	69.08±1.52	80.00±1.08	0.41±0.00		
	24H	0.0512±0.00	0.0362±0.00	0.0877±0.00	1.4168±0.05	0.0380±0.00	64.75±1.35	77.59±0.53	0.27±0.00		
	48H	0.0463±0.00	0.0339±0.00	0.0803±0.00	1.3655±0.01	0.0322±0.00	62.08±0.11	77.27±0.75	0.18±0.00		
	96H	0.0388±0.00	0.0318±0.00	0.0707±0.00	1.2201±0.01	0.0266±0.00	50.66±2.56	65.40±0.78	0.15±0.00		
AR	12H	0.0485±0.00	0.0434±0.00	0.0922±0.00	1.1175±0.02	0.0276±0.00	65.40±0.89	74.95±1.06	0.32±0.00		
	24H	0.0350±0.00	0.0302±0.00	0.0653±0.00	1.1610±0.03	0.0210±0.00	60.62±0.48	72.80±0.14	0.19±0.00		
	48H	0.0316±0.00	0.0246±0.00	0.0563±0.00	1.2851±0.03	0.0198±0.00	56.31±0.24	70.85±0.41	0.13±0.00		
	96H	0.0266±0.00	0.0208±0.00	0.0477±0.00	1.2788±0.01	0.0160±0.00	47.02±1.50	61.03±3.46	0.09±0.01		
Gaucho + Krilaxyl	12H	0.0306±0.00	0.0360±0.00	0.0516±0.00	0.8500±0.03	0.0172±0.00	73.80±3.68	71.60±0.72	0.23±0.00		
	24H	0.0197±0.00	0.0286±0.00	0.0451±0.00	0.6926±0.06	0.0151±0.00	70.67±0.70	69.83±0.18	0.16±0.00		
	48H	0.0185±0.00	0.0206±0.00	0.0391±0.00	0.8985±0.00	0.0120±0.00	52.56±0.63	66.90±0.59	0.10±0.00		
	96H	0.0109±0.00	0.0188±0.00	0.0299±0.00	0.5797±0.03	0.0088±0.00	44.62±3.18	51.56±4.70	0.05±0.00		
CONTROL	12H	0.0865±0.00	0.0986±0.00	0.1851±0.00	0.8772±0.02	0.0602±0.00	87.00±2.61	98.84±2.56	1.18±0.02		
	24H	0.0810±0.00	0.0906±0.00	0.1717±0.00	0.8949±0.03	0.0560±0.00	82.93±0.08	95.99±0.86	0.63±0.01		
	48H	0.0665±0.00	0.0703±0.00	0.1370±0.00	0.9486±0.05	0.0415±0.00	76.12±0.10	91.54±0.74	0.46±0.00		
	96H	0.0615±0.00	0.0634±0.00	0.1240±0.00	0.9700±0.02	0.0345±0.00	69.36±3.74	84.01±1.06	0.41±0.02		

CHL : Chlorophyll, TOT CHL : Total Chlorophyll, RWC : Relative Water Content, CAR : Carotenoid, WT : Weight, AM : Arithmetic Mean, SD : Standard Deviation. EP : Exposure Period, H : Hour, BR : Below Recommended, RD : Recommended Dosage, AR : Above Recommended, I+F : Insecticide + Fungicide, Value are represented as Arithmetic mean and Standard Deviation of four determinants.

Anderson¹¹, Turner and Marshal¹², Chiou and Muller¹³ respectively. All the tabulated data were analyzed using Arithmetic mean and standard deviation of four determinants¹⁴.

Results and Discussion

Effect on Percent Germination (table 1) : The percent germination recorded after 12, 24, 48 and 96 hrs in responses to Krilaxyl and Gaucho treatment. The response of seed germination to both the pesticidal treatments was severely affected at higher and in combination treatment of all the exposure periods. This reduction in the seed germination was due to inhibitory action of both Krilaxyl and Gaucho on metabolic activities. As compared to the untreated seeds, germination was greatly reduced and delayed by long-term treatment of higher dose of both the pesticides (48 and 96 hrs.). This was due to their injurious action which disturb the osmotic relation of the seed and thereby reduced seed germination^{15,16}.

However, the higher and in combination dosages of all the exposure periods produced much variation and delayed the germination. The percent of germinating seeds were affected and further growth decreased during long-term treatment. But, the lower concentration of lower exposure periods did not give much change on germination¹⁷.

Effects on Early Seedling growth (Table 1) : As the concentrations and duration of the treatment was increased, the seedling growth was found to be affected. This growth inhibition of root and shoot at higher and in combination treatment during all the exposure periods indicates the toxic effects of both the pesticides on cell division of shoot and root meristem as well as elongation.

The ratio of root and shoot decreases as the dose of pesticides increased suggesting, an inhibition of root development. This indicates that, these concentrations accelerated the growth of plumule, while affecting the growth of radicle adversely¹⁸⁻²⁰.

Effects on Chloroplast Pigments (Table 2) : Chlorophyll content were greatly reduced at longer (48 & 96 hrs) exposure periods of all the concentrations by both the pesticides as compared to control²¹. Even though, there is an influence in increase of chlorophyll 'b' over chlorophyll 'a' at higher dose of Gaucho and in combination dosage of both the pesticides at all the exposure periods have been recorded.

Some of the possible reasons for the decrease in chlorophyll content may be due to the formation of enzyme i.e, chlorophyllase, which is responsible for chlorophyll degradation²²⁻²⁴. Further, the Carotenoides decreases only at longer exposure periods of all the concentrations of Krilaxyl and combined treatments. In case of Gaucho, it increased at longer exposure periods

of all the concentrations.

Effects of Relative Water content (Table 2) : With the increase and in-combination dosages of long-term treatment, the relative water content of both leaves and seedlings were significantly decreased, in both the treatments as compared to control. This decrease of relative water content in leaves and seedlings was due to toxicity of both the pesticides on membrane permeability and increased resistance to water flow in the system. The level of phytotoxicity was higher with increased and in-combination dosages of all the exposure periods and thereby affected the growth to greater extent (table 1).

Similarly, vigour index and tolerance index (Table 1) were also decreased with increase in concentrations of all the exposure periods over control. Further, the fresh weight and dry weight (Table 1&2) also showed decline at higher and in-combination dosages of all the exposure periods²⁵.

To conclude, it is revealed that, the variation in the concentrations of Krilaxyl and Gaucho, singly and in-combinations during long term treatment had the inhibitory effects on germination, early growth, pigment content and other parameters of Rice cv. Mangala^{3,26}. But, lower dosages of lower exposure periods did not induce much change on germination and early seedling growth. In light of the present study, the higher doses of longer exposure periods are not beneficial on germination and subsequent growth of above cereal crop. Hence, the farmers are advised to use only the advocated doses of pesticides to their respective crop plants.

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