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4 **Enhancing Planting Value of Rice Seed through**
5 **Priming with Humic Substance**

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8 **ABSTRACT**
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A study was carried out at seed testing laboratory of Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan to examine the response of rice to treatment with Plant Growth Regulator (Vimpel™) developed in Nigeria, with a view to determining the effect of the humic substance on germination and seedling growth rate of the seed. FARO 44 rice variety was divided to five parts before priming each lot with four varied concentrations: (0%, 25%, 50% and 100%) and one control (dry unprimed seed). Fifty seeds in three replicates were drawn from each concentration after two (2), four (4) and eight (8) hours of priming (duration) and planted into separate planting round transparent plastic bowl filled with sterilized river sand. Each treatment was replicated three times and the experiment was repeated twice. Data was collected on final germination percentage (FGP), mean germination time (MGT), coefficient of velocity of germination (CVG), seedling vigour index (SVI), germination rate index (GRI), relative seedling growth rate (RSGR) and speed of germination index (SGI). Rice seed priming with PGR (Vimpel™) did not significantly improve seed germination but the growth rate is significantly influenced with priming with humic substance. Priming with 50% concentration of humic substance for 4 hours significantly improve the seedling growth rate of rice. Therefore, priming rice seed with humic substance can improve seedling growth and vigour of rice under upland and lowland cultivation.

10
11 *Keywords: Rice seed, Priming, Humic substance, Seedling growth.*

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13 **1. INTRODUCTION**
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15 Rice (*Oryza sativa*) is the world's most important staple food in Africa and Latin America [1]. Its
16 global production has been estimated to be at the level of 650 million tones and the area under
17 cultivation have been estimated at 156 million hectares [2]. Rice is known as semi aquatic,
18 annual grass plant and grows in a wide range of soil and water regime: irrigated, rain fed
19 lowland, upland and flood prone. Peasant farmers are the largest producers of food consumed
20 in most developing nations including Nigeria; however, most of these farmers are resource poor
21 and unable to engage in profitable agriculture that can sustain food sufficiency in those
22 countries. These farmers have been adopting various means to boost their agricultural
23 production. Rice requires some important nutrients to grow effectively for higher yield [3]. A
24 common approach to supply the nutrients to the crop is through fertilizer application because of
25 varying levels of soil degradation and fertility. About 70% of the farmers agreed that the use of
26 fertilizer can lead to increased yield [4], however, inaccessibility of farmers to chemicals and
27 fertilizers due to cost and availability has been a major concern to rice farmers. Also, continuous
28 use of chemical fertilizer has been reported to have deleterious effect on soil [5]. It therefore
29 becomes necessary to seek a more sustainable and cost effective approach to supply these
30 nutrients to rice plant. Seed treatment through priming has been identified as an attractive
31 approach to enhance crop establishment [6]. Treatment of seeds with a variety of inorganic

32 and/or organic compounds, some of which are synthetic, has been successfully demonstrated
33 to improve germination and seedling establishment in seeds of many field crop such as wheat,
34 soybean, sunflower and maize [7]. Also, [8] reported that seed priming of maize, wheat, rice and
35 canola resulted into better germination and establishment. [9] concurred that treatment of seeds
36 with the right products has the potential to improve seedling emergence and establishment as
37 well as plant stands. Humic substances are “end product” of decaying organic matter. They
38 have positive effects on plant physiology by influencing nutrient uptake and root architecture
39 [10]. Humic substances can be likened to bio-stimulants which are non-fertilizer products that
40 have beneficial effect on plant growth. The European Bio-stimulants Industry Council (EBIC)
41 described plant bio-stimulants as substances that contains micro-organisms that stimulate
42 natural processes to enhance/benefit nutrient uptake, nutrient efficiency, when applied to plants
43 or the rhizosphere in small quantities [11]. There are commercial bio-stimulants and inorganic
44 products that are claimed by the manufacturers to play an important role in complementing plant
45 morphological and physiological growth [12].

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47 Since, there is elevated interest in finding alternative measures to manipulate either seed
48 germination or seedling growth or both in an attempt to ensure there is an optimum plant stand
49 with vigorous vegetation growth. This study, therefore examined the seedling growth response
50 of rice to seed priming with humic substance (Vimpel™) with the objectives of determining the
51 appropriate concentration and period of priming that will enhance germination, seedling vigour
52 and growth rate of rice.

53 54 55 **2. MATERIAL AND METHODS**

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57 The study was carried out in Seed Testing laboratory of Institute of Agricultural Research and
58 Training, Ibadan, Nigeria. One kg seed of FARO 44 rice variety, sourced from AfricaRice
59 Centre, Ibadan was first divided into two equal parts. Each halve was then divided into five parts
60 of 100g each, with each part representing varied Vimpel concentrations: 0% (100ml of distilled
61 water only), 25% (25mls Vimpel+75mls of distilled water), 50% (50mls of Vimpel +50mls of
62 distilled water), 100% (100mls of Vimpel only) and Control (Dry unprimed seed). Fifty seeds in
63 three replicates were drawn from each concentration after two (2), four (4) and eight (8) hours of
64 priming (duration) and planted into separate planting round transparent plastic bowl filled with
65 sterilized river sand in three replicates. The river sand was adequately moistened before
66 planting and regularly on daily basis. The bowls were arranged using Completely Randomized
67 Design (RCD) in the seed testing laboratory and allowed to grow under ambient environment of
68 24°C and relative humidity of 63%. Each planting bowl represented each replicate and the
69 experiment was conducted twice. Data were collected on germinated seedlings as follows:

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71 i. Germination count: Number of seedlings germinating each day was counted on daily
72 basis starting from the date of first emergence (4 days) to 13 days after planting when
73 there was no more germinating seed
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75 ii. Shoot length: Ten randomly selected seedlings were tagged using paper tape and
76 numbered in each bowl without bias. The length was measured from the base of the
77 shoot to top of the crop on daily basis using transparent ruler and recorded every of
78 other day beginning from 7 days after planting.

79 Data collected were used to estimate the following seed germination and seedling vigour
80 characters:

- 81 i. Final germination percentage (FGP) was determined by finding the ratio of normal
82 germinated seed at 13 days after sowing to total number of seeds planted. This is in
83 according to the method suggested by [13]:
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$$FGP = \frac{\text{Number of germinated seedling at final day}}{\text{Number of seed planted}} \times 100$$

- ii. Mean Germination Time (MGT): This represents the mean time a seed lot requires to initiate and end germination.
- iii. Coefficient of Velocity of Germination (CVG) : This is an estimate of the rapidity of germination of the seed lot and it was estimated according to the method described by Scott *et al*, (1984) :

$$CVG = \frac{\sum Ni}{\sum NiTi} \times 100$$

where: N is the number of seeds germinated each day and T is the number of days corresponding to N

- iv. Seedling Vigour Index (SVI): It was evaluated on the fourteenth day of planting using the formula of [14] based on the product of germination (%) and seedling length at the 14th day after planting as follows:

$$SVI = \frac{(\text{Germination \%} \times \text{Seedling length})}{100}$$

- v. Germination rate index (GRI): This gives an indication of the percentage of seeds germinating each day of the germination period. It is calculated as

$$GRI = \frac{G_1}{1} + \frac{G_2}{2} + \frac{G_3}{3} + \dots \dots \dots \frac{G_x}{x}$$

where G1 = Germination percentage x 100 at the first day after sowing,
G2 = Germination percentage x 100 at the second day after sowing

- vi. Relative Seedling Growth Rate (RSGR): This is a measure of the increase in seedling growth over a period of time. It is measured as the mass increase in the shoot length above soil level per day from the onset of the measurement till the termination of the shoots and calculated by modifying the equation described by [15] for crop growth rate as

$$RSGR = \frac{(H_2 - H_1)}{(D_2 - D_1)}$$

where H1 = Plant height (cm) recorded at time D1,
H2 = Plant height (cm) recorded at time D2,
D1 and D2 were the interval of days respectively (cm/day)

- vii. Speed of germination index (SGI): Speed of germination index was calculated as described by [16]AOSA (2003) :

$$SGI = \frac{\text{Number of germinated seeds at Day 1} + \dots + \text{Number of germinated seed at the last day}}{\text{Days of first count at Day 1} + \dots + \text{days of final count}}$$

Data obtained were subjected to combined analyses of variance (ANOVA). Difference between the treatments was separated using Duncan Multiple Range Test (DMRT) at 5 % or 1 % levels of significance. Pearson's coefficient of correlations between pairs of seed germination indices and seedling growth characters were determined using [17].

3. RESULTS

Analysis of Variance for Seed Germination Indices and Seedling Growth Characters

Combined analysis of variance (ANOVA) revealed highly significant difference between the experiments (E) for all the seed germination indices: final germination percentage (FGP) and

135 seedling growth parameters: mean germination time (MGT), coefficient of velocity of
 136 germination (CVG), seedling vigour index (SVI), germination rate index (GRI), relative seedling
 137 growth rate (RSGR) and speed of germination index (SGI)] at $P < 0.01$ (Table 1). Also, there
 138 were highly significant differences in the response of the rice seed to varied concentration of
 139 humic substance (C) used for priming. The duration of priming (D) had high significant effects
 140 on all of the characters measured. Interaction of the humic concentrations (C) and duration of
 141 priming (D) revealed significant interactive effects on most of the characters measured between
 142 the experiments, whereas interaction between the experiments (E) and duration of priming (D)
 143 as well as interaction between the experiments (E), concentration of humic substance (C) and
 144 duration of priming (D) were not significant for all of the traits studied. The interaction between
 145 concentration of humic substance (C) and duration of priming (D) only had significant effects on
 146 MGT and RSGR in this study (Table 1). Coefficient of variation (CV) ranged from 3.29% for
 147 MGT to 21.47% for RSGR.

149 **Table 1: Combined Analysis of variance (ANOVA) for seed germination indices and**
 150 **seedling growth characters of rice as affected by priming with humic substance**
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SV	df	FGP	MGT	CVG	SVI	SGI	GRI	RSGR
Experiments (E)	1	1472.18**	1.56**	375.03**	224.05**	50884.44**	2653.32**	1.26**
Concentration(C)	4	26.73	0.87**	7.16	22.76**	3465.78**	300.15**	1.71**
Duration (D)	2	659.24**	0.84**	102.08**	37.51**	11764.21**	223.02**	3.67**
E x C	4	35.18	0.80**	6.51	7.25	3902.33**	309.99**	0.31*
E x D	2	32.31	0.04	15.48	0.92	931.01	33.05	0.02
C x D	8	75.30	1.33**	18.24	5.48	1209.54	27.12	0.84**
E x C x D	8	205.64	0.04	37.22	13.44	4413.73	107.06	0.09
Pooled error	60	41.96	0.04	11.16	3.47	849.69	21.74	0.11
Mean		83.96	5.98	18.80	15.15	378.89	55.64	1.53
Minimum		60.00	4.09	9.00	8.47	266.00	37.50	0.10
Maximum		100.00	7.17	30.50	22.42	464.00	78.09	2.80
SE(m)		0.99	0.05	0.47	0.31	5.02	1.00	0.06
CV%		7.72	3.29	17.77	12.30	7.69	8.38	21.47

152 *, ** Significant at ($P = 0.05$) and ($P = 0.01$) respectively

153 SV: Source of variation; df: degree of freedom; SE(m): Standard error of mean; CV: Coefficient
 154 of variation; FGP: Final germination percentage; MGT: Mean germination time; CVG: Coefficient
 155 of velocity of germination; SVI: Seedling vigour index; GRI: Germination rate index; RSGR:
 156 Relative seedling growth rate; SGI: Speed of germination index

161 **Seed germination indices and seedling growth characters of rice as affected by**
 162 **priming with varied concentrations of humic substance**
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164 The final germination percentage (FGT) and coefficient of velocity of germination (CVG) were
 165 not significantly affected by the concentrations of the humic substance (Table 2). Although,
 166 there was no significant difference in the Mean germination time (MGT), Seedling vigour index
 167 (SVI) and relative seedling growth rate (RSGR) of rice seed primed with 0%, 25% and 50%
 168 concentrations of humic substance, there were corresponding increase in the MGT and SVI as
 169 priming concentrations increases, with seed primed with 100% concentration of the humic

170 substance recorded significantly higher MGT (62) and SVI (16.48), when compared with other
 171 concentrations and the unprimed seed. Conversely, the germination rate index (GRI) and
 172 seedling germination index (SGI) decreases as the priming concentration increases (*Table 2*).
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174 **Table 2: Effect of priming rice seed with varied concentration of humic substance on**
 175 **seed germination indices and seedling growth rate of rice seed.**
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Concentration	FGP	MGT	CVG	SVI	SGI	GRI	RSGR
Control (Un-primed)	85.33a	5.62c	18.54a	13.50c	401.50a	62.45a	1.09c
0%	85.22a	6.06b	19.22a	15.61ab	380.72b	55.19b	1.49b
25%	82.89a	5.98b	17.81a	15.49ab	373.89b	54.97b	1.57b
50%	85.34a	6.02b	19.31a	14.67ab	373.72b	54.15ab	1.51b
100%	83.00a	6.22a	19.14a	16.48a	364.61b	51.46c	1.96a

177 *Mean followed with same alphabet along the same column are not significantly different*
 178 *from each other at 5% significant level*
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 181 **Seed germination indices and seedling growth characters of rice as affected by duration**
 182 **of priming with humic substance**
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184 The FGP, SVI, SGI and GRI of rice seed primed with humic substance for four and eight hours
 185 were not significantly different from each other. However, rice seeds primed for 8 hours were
 186 consistently recorded higher value for FGP, SVI, SGI, GRI and RSGR. All the seed germination
 187 indices and seedling growth except CVG increased as duration of priming increases from 2 to 4
 188 hours. (*Table3*).
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 191 **Table 3: Seed germination indices and seedling growth rate of rice seed as affected by**
 192 **duration of priming with humic substance**
 193

Duration	FGP	MGT	CVG	SVI	SGI	GRI	RSGR
2 hours	78.73b	5.83c	16.94c	13.92b	356.70b	52.50b	1.40b
4 hours	85.33a	5.95b	20.63a	15.43a	385.20a	57.09a	1.25b
8 hours	87.80a	6.16a	18.84b	16.10a	394.77a	57.34a	1.92a

194 *Means with the same letter(s) in the same column or row are not significantly different from*
 195 *each other at P = 0.05*
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197 *FGP: final germination percentage; MGT: mean germination time; CVG: coefficient of velocity of*
 198 *germination; SVI: seedling vigour index; GRI: germination rate index; RSGR: relative seedling*
 199 *growth rate; SGI: speed of germination index*

200 **Variation of seed germination indices and seedling growth characters measured in the**
 201 **two experiments**

202 The mean of seed germination indices and seedling growth characters across the treatments
 203 (duration of priming and concentration regimes) shows that Experiment 1 had higher overall
 204 mean values than Experiment 2 for all of the characters measured in this study except MGT
 205 (*Table 4*).
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Table 4: Seed germination indices and seedling growth parameters measured as affected by frequency of experiments

Traits	Experiment	Mean	Std. Error
Final germination percentage	1	88.00a	0.966
	2	79.91b	0.966
Mean germination time	1	5.851b	0.029
	2	6.11a	0.029
Coefficient of velocity of germination	1	20.84a	0.498
	2	16.76b	0.498
Seedling vigour index	1	16.72a	0.278
	2	13.57b	0.278
Speed of germination index	1	402.67a	4.345
	2	355.11b	4.345
Germination rate index	1	61.07a	0.695
	2	50.22b	0.695
Relative seedling growth rate	1	1.644a	0.049
	2	1.408b	0.049

212 *Means with the same letter(s) in the same column or row are not significantly different from*
213 *each other at P = 0.05*

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Pearson Correlation between pairs of seed germination indices and seedling growth characters

217 The Pearson correlation coefficient between pairs of seed germination indices and seedling
218 growth characters across the experiments are presented in Table 5. FGP was positively and
219 highly significantly associated with CVG (0.91**), SVI (0.72**), GRI (0.80**) and SGI (0.95**).
220 Also, MGT was positively and significantly correlated with RSGR (0.61**), but significantly and
221 negatively associated with SGI (-0.35**) and GRI (-0.49**). It was also observed that CVG was
222 positively and significantly correlated with SVI (0.70**), SGI (0.82**) and GRI (0.65**). A positive
223 and significant correlation was also observed between SVI and SGI (0.60**), GRI (0.46) and
224 RSGR (0.65**) as well as between GRI and SGI (0.95**).

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Table 5: Pearson coefficient of correlation between pairs of seed germination indices and seedling growth characters across the experiments

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	FGP	MGT	CVG	SVI	SGI	GRI	RSGR
FGP	-	-0.11	0.91**	0.72**	0.95**	0.80**	0.12
MGT		-	0.01	0.15	-0.35**	-0.49**	0.61**
CVG			-	0.70**	0.82**	0.65**	0.18
SVI				-	0.60**	0.46**	0.65**
SGI					-	0.95**	0.00
GRI						-	-0.04
RSGR							-

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**, ** Significant at (P= 0.05) and (P= 0.01) respectively*

230 *FGP: Final germination percentage; MGT: Mean germination time; CVG: Coefficient of velocity*
231 *of germination; SVI: Seedling vigour index; GRI: Germination rate index; RSGR: Relative*
232 *seedling growth rate; SGI: Speed of germination index*
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234 **4. DISCUSSION**

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236 Seed germination depends on both external and internal factors around the seed [18]. The
237 significant response of rice seed to varied concentration of humic substance and duration of
238 priming suggests that seed germination indices and seedling growth characters of rice is
239 dependent on many factors. The germination rate index (GRI) and seedling germination index
240 (SGI) that decreased as the priming concentration increases in this study agrees with the finding
241 of [19] who reported that with increasing concentrations of plant growth regulator, seedling
242 growth and germination percentage of cowpea traits decreased dramatically, while other
243 characters (SVI and RSGR) were inconsistent as the concentration increases. Comparatively,
244 there was no significant difference between control (no treatment) and priming concentration
245 regimes for FGP and CVG. This implies that the rapidity of germination of the seed lot was not
246 improved by the priming treatment as none of the treatment performed significantly better than
247 the control. This observation corroborates the work of previous scientists [20, 21] who reported
248 that plant growth regulator (ComCat™) concentrations did not have significant effect on
249 coleoptile growth of maize compared to the untreated control. On the other hand, it was
250 observed that the seed lot without priming (control) had significantly low values as compared to
251 the primed seed lot for MGT, SVI and RSGR. This suggests that seed treatment with humic
252 substance can be used to improve the seedling vigour during seedling growth stage.

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254 The GRI reflects the percentage of germination on each day of the germination period. Higher
255 GRI values indicate higher and faster germination [22]. The GRI and SGI of rice seed lot that
256 was not primed had better performance than primed seed lot. However, highest means
257 recorded in MGT, SVI and RSGR of rice seed primed with 100% concentration agrees with the
258 result of [23] who reported that priming of seeds with low concentration of Gibberellic Acid (GA₃)
259 had no effect on seed germination while higher concentration of GA₃ increased shoot and root
260 lengths, dry weight, fresh weight and tissue water content of maize.

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262 It can be deduced that 4 hours of priming favoured most of the seed germination indices and
263 seedling growth traits studied. This indicates priming rice seeds for 4 hours significantly
264 improved most of the characters in this study. This shows that seed germination indices and
265 seedling growth of rice can be improved by the duration of priming or seed soaking time,
266 because four hours priming resulted in higher mean values for most of the characters in this
267 study. [24] reported stimulatory effects of priming on the early stages of germination process
268 with mediation of cell division in germinating seeds. Priming may improve germination by
269 accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication
270 of radicle cells [25]. It can be deduced that 4 hours of priming favoured most of the seed
271 germination indices and seedling growth traits in this study

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273 The significant difference observed in the reaction of the rice seed to humic substance priming
274 in the two experiments shows that the first experiment had higher overall mean values that were
275 significantly different from the second experiment for all of the characters measured in this study
276 (FGP, CVG, SVI, GRI, RSGR and SGI) except MGT. However, the trend of the result in the
277 result obtained in the two experiments were similar, hence the situation may be attributed to
278 both internal and external environmental factors during the conduct of the experiments and may
279 not be necessarily due to reaction of the seeds to the treatment.

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281 Simple correlation analysis has been considered adequate as a rough guide to the magnitude
282 and direction of the relationships between two traits [14]. Therefore, positive and significant

283 correlation observed among the pairs of seed germination indices and seedling growth
284 characters in this study suggests that these characters can aid in selection during rice
285 improvement programs. [14] reported that significant positive correlation indicates that selection
286 for one character could be used to indirectly select for another character. This study showed
287 that seeds with high germination value will positively influence the other seedling growth
288 parameters.

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290 5. CONCLUSION

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292 Rice seed priming with PGR (Vimpel™) did not significantly improve seed germination but the
293 growth rate is influenced with the priming. Rice seed priming at 50% concentration for 4 hours
294 significantly improve the seedling growth rate of rice. This study, therefore indicate priming rice
295 seed with humic substance will improve seedling growth and vigour of rice under upland and
296 lowland cultivation

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300 COMPETING INTERESTS

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302 No competing interests exist.

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