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Influence of Fungicidal Seed Treatments against Pathogen Complex Found on Paddy Seed and Their Effects on Seedling Germination, Growth, and Plant Vigor

Rajendra Persaud[1], Jomeala Gilkes[1], Danata McGowan[1], Narita Singh[2], Darshanie Angela Persaud[3], Elroy Charles[4] & Gomathinayagam Subramanian[5]

[1] Guyana Rice Development Board (GRDB), Rice Research Station (RRS), Burma, Mahaicony, East Coast Demerara, Guyana

[2] School of Allied Health, University of Guyana, Turkeyen Campus, Greater Georgetown, Guyana

[3] The Faculty of Earth and Environmental Sciences, Department of Environmental Studies, University of Guyana, Turkeyen Campus, Greater Georgetown, Guyana

[4] Faculty of Agriculture and Forestry, University of Guyana, Turkeyen Campus, Greater Georgetown, Guyana

[5] Faculty of Agriculture and Forestry, University of Guyana, Berbice Campus, Tain, Guyana

Correspondence: Rajendra Persaud, Guyana Rice Development Board (GRDB), Rice Research Station (RRS), Burma, Mahaicony, East Coast Demerara, Guyana. E-mail: rajpersaud25@yahoo.com; rpersaud@grdb.gy

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Abstract

A paddy (*Oryza sativa*) seed treatment study was conducted by the Plant Pathology Department, GRDB, RRS, Burma to evaluate the effectiveness of eight fungicides [Amistar Xtra 28SC, Saaf 75 WP, Glory 75WG, Tridium 70WG, Antracol 70WP, Rodazim 50 SC, Carbendazim 50SC and Manzate Pro Stick TM], at varying rates, along with a healthy check and untreated control, on germination and seedling vigour of paddy, and identifying suitable fungicides to be used as pre-sowing seed treatment. Trials were carried out under *laboratory, potted* and *field conditions*. A completely randomized block (CRD) was used for the *laboratory* and *pot trials* and randomized complete block design (RCBD) was used for the *field trials*, with three replicates. These trials were conducted in both rice growing seasons (Spring and Autumn) in Guyana during 2020 and 2021. Dry seed examination and blotter test unearthed the presence of four dominant pathogenic genera (*Curvularia lunata, Bipolaris oryzae, Pyricularia oryzae* and *Aspergillus* spp). from the paddy seeds before being treated and in the untreated control. The results of the germination test during 2020 showed variability among treatments, percentage germination ranged from 61% in seeds treated with Amistar Xtra 28 SC 3.7ml/L to 91% in seeds treated with Saaf 75 WP 5.6g/L. While in 2021 the percentage germination ranged from 79% in seeds treated with Tridium 70 WG 4.3g/L to 95% in seed treated with Saaf 75 WP 5.6g/L. Results from the *in vitro* studies showed seeds treated with Saaf (3.1g/L and 5.6g/L), Manzate Pro Stick TM (3.7 g/L), Glory at 12.4g/L, Rodazim (at 3.7ml/L) and the healthy control produced seedlings with taller shoots, longer roots and ultimately the higher seedling vigour at 14 and 21 days after treatment as compared to other treatments and the untreated control. Similarly, under *potted* conditions, seed treated with Rodazim (1.2ml/L and 3.7ml/L), Saaf (5.6g/L) and Glory (7.4g/L and 12.4g/L) demonstrated higher SVI compared to the other treatments and the untreated control. Further, under *field* condition the three fungicides *viz.* Rodazim, Manzate and Glory found to be promising in previous trials were selected and evaluated against an untreated control. Data collected revealed no significant difference among any of the treatments for grain yield, number of tillers/m^2, plant height, the weight per 1000 grain nor the number of unfilled grains per panicle. However, treatment with Manzate Pro Stick TM (3.7 g/L) produced the largest number of filled grains as compared to the other treatment and control. It is therefore recommended that farmers should employ seed treatment prior to sowing if do not have clean, certified disease free seeds and use the paddy seed treatment [Saaf (5.6g/L), Manzate Pro Stick TM (3.7 g/L), Glory at (12.4g/L), Rodazim (3.7ml/L)] as a method of controlling seed borne pathogens, enhancing germination, seedling vigour and ultimately improving productivity.

Keywords: fungicide, seed treatment, pathogen, rice, germination, seedling vigor

1. Introduction

Rice has become the most economically important food crop in many countries due to food diversification (Persaud et al., 2023). The increase in rice productivity has become necessary in order to meet the world's current population growth rate (Ou, 1985); many diseases, however, affect the rice crop and decrease yield considerably (GRDB, 2022). The rice industry in Guyana is currently the largest agricultural industry with approximately six thousand (6,000) families directly and one hundred and fifty thousand (150,000) persons indirectly associated with the industry (GRDB, 2023). In Guyana, rice diseases continue to be an important factor limiting profitable rice production and are considered to be the main biotic and abiotic factors that affect rice cultivation productivity (Persaud et al., 2021; Ou, 1985; Chouhan and Kumar, 2022). Among them are the major diseases; Rice Blast (*Pyricularia oryzae*)*;* Brown spot (*Bipolaris oryzae* (synonyms *Dreschlera oryzae* and *Helminthosporium oryzae*)*;* Sheath Blight (*Rhizoctonia solani*) and Sheath rot (*Sarocladium oryzae*). In addition, false smut (*Ustilaginoidea virens)* and grain discoloration (various microorganisms associated) can be considered as minor diseases (GRDB, 2022).

Every seed is a potential harbor of a wide variety of mycoflora containing both pathogenic and saprophytic microorganisms, both externally and internally (Utobo et al., 2011). Infested seeds are the primary inoculum sources to spread many diseases (Miah et al., 2017). These mycoflora deteriorate seed quality, affect viability and reduce germination of seeds, resulting in the production of abnormal seedlings (Paul, 1989 and Khanzada et al., 2002).

Sowing seed of high quality is necessary for small scale farmer's crop production. Poor quality seeds will result in low field emergence and in seedlings that are less tolerant to abiotic stress, more sensitive to plant diseases and will reduce the quality and yield of crops produced (Ahmed and Siddiqui, 1995). Seed mediated disease transmission plays a greater role in disease spread, the pathogen which harbours on rice seed is suitable inoculum for disease development in the rice field (Utobo et al., 2011). Seed treatment using fungicides plays a vital role in the management of seed-borne pathogens (Naher et al., 2016). The actual impact and the economic benefits are still not yet clear and need to be further investigated. Also, the best suitable fungicides and the correct rate of application is still unknown. Seed treatments also reduce the environmental impact of the production process by decreasing the number of spray applications of agrichemical products and lessening exposures to non-target species including humans and pollinators (Filippi and Prabhu (1997).

Seed mediated disease transmission plays a great role in the spread of disease (Amgai et al., 2012; Taye et al., 2013). Thus, considering the aforementioned, the present trial was conducted to determine what effects the use of fungicides as seed treatment would have on germination, growth and seedling vigour of rice - *Oryzae sativa* L under Guyana's agricultural conditions.

2. Materials and Method

Site of trial: This experiment was conducted at the Rice Research Station, Burma of the Guyana Rice Development Board, Burma, East Coast Demerara, Guyana under laboratory, pots culture and field conditions during 2020 and 2021.

Seed Health Testing: Seeds from a disease susceptible rice variety (Rustic) were collected and evaluated *via* standard blotter method for the presence of fungal pathogen. Seeds were incubated for seven days under controlled lighting conditions (12 hours light and 12 hours of darkness), after which microscopic observations were made to determine the fungal pathogen present. Seeds to be used as the healthy control were collected from visibly healthy plants and pre-surface sterilized using 0.5% sodium hypochlorite solution to remove any unwanted microorganisms present externally on the seed surface and store to be use in this experiment.

Laboratory Experiment: Seventy-five seeds of each treatment along with an untreated control and a healthy control were carefully counted, treated, and placed into three petri dishes on filter paper. Twenty-five of the treated seeds were placed in each petri dish and kept under 12 hours lighting and 12 hours darkness for incubation. Distilled water was added as required to maintain the seeds and filter papers moist. The experiment was conducted using a Complete Randomized Design (CRD) with three replications for each treatment and the untreated and healthy control. For the laboratory studies eight fungicides at various rates were evaluated against an untreated and healthy control (Table 1).

Pot culture trial*:* Treated, pre-sterilized and untreated seeds were sown in pots. Each pot was filled with an equal amount of soil and twenty-five seeds were sown per pot. Water was added as required. The experiment was conducted in a CRD with three replications for treatment and control. In the pot culture trial eight fungicides at various rates were evaluated against an untreated and healthy control (Table 1).

Field experiment: The experimental design utilized was a Randomized Complete Block Design RCBD for the field evaluations with three replications. Seeds from susceptible variety (Rustic) were used. In the case of the field evaluation three of the better preforming fungicides evaluated under the laboratory and pot culture trial in 2019 were selected and evaluated against an untreated control under field conditions. Fertilizer application, early season pest control, weed management follow the standard crop production practices as recommended and described by GRDB.

Method of Seed Treatment: Eight fungicides *viz.* Amistar Xtra 28SC, Saaf 75 WP, Glory 75WG, Tridium 70WG, Antracol 70WP, Rodazim 50 SC, Carbendazim 50SC and Manzate Pro Stick TM, at varying rates (Table 1) were evaluated against a pre sterilized (healthy control) and an untreated control. Seeds were mixed thoroughly in each treatment to facilitate proper coating while the seeds to be used as the healthy control were treated with 0.5% sodium hypochloride solution.

Table 1. List of fungicides and treatments

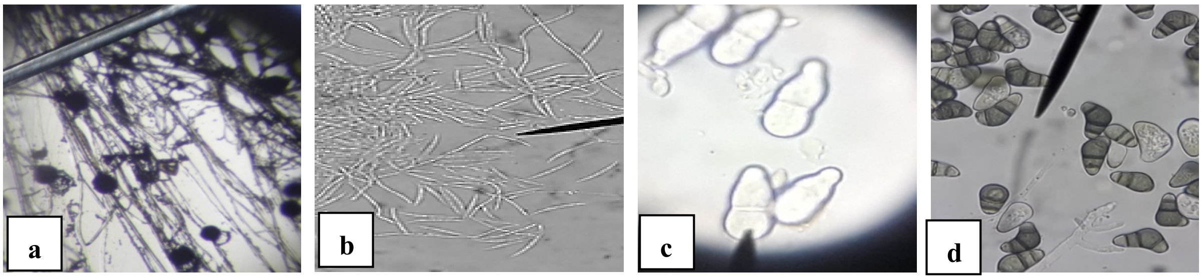
Trt	Chemicals	Active ingredients	Rates
T1	**Amistar Xtra 28 SC**	Triazol, Estrobilurtina., Cyproconazol, Azoxystrobin	2.5 ml/L
T2			3.7 ml/L
T3	**Saaf 75 WP**		4.0g//L
T4			5.6g//L
T5	**Glory 75 WG**	Mancozeb + Azoxystrobin	7.4 g/L
T6			12.4 g/L
T7	**Tridium 70 WG**	Azoxystrobin 4.7% + Mancozeb 59.7% + Tebucuzonal 5.6% WG	3.1g/L
T8			4.3g/L
T9	**Antracol 70WP**	Propineb	6.2g/L
T10	**Rodazim 50 SC**		1.2ml/L
T11	**Rodazim 50 SC**		3.7ml/L
T12	**Carbendazim 50SC**	Carbendazim 50%	3.7ml/L
T13	**Manzate Pro Stick TM**	Mancozeb 70%	3.7g/L
T14	**Non-Treated Control**	Distilled water	-
T15	**Healthy control**	(Pre-sterilize)	-

Data Collection: Seeds of each treatment were subjected to assess the germination and seedling vigour as per procedure recommended by ISTA. Seed germination was recorded seven days after treatment was applied based on number of seeds germinated out of total number of seeds set. The germination percentage computed, then the root length and shoot length recorded a t14 and 21 days after treatment. Ten plants in each replication were random selection for data collection. Seedling vigor index (SVI) at 14 and 21 days after sowing (DAS) was calculated as per formula described by Abdul Baki and Anderson (1973): SVI = (mean root length + mean shoot length)* percent germination.

Statistical analysis: The variance of data was analysed using analysis of variance (ANOVA) with Statistix 8 software, and mean values for each trait were compared according to Least Significant Difference (LSD) statistical test. Charts and graphs derived from using Microsoft Excel 2017.

3. Results and Discussion

Seed examination and standard blotter test: Several seed associated mycoflora were observed on seeds collected from the disease susceptible rice variety (Rustic) before the seeds were treated and in the untreated control. The predominant genera of microorganisms observed and identified were *Curvularia lunata, Bipolaris oryzae, Pyricularia oryzae* and *Aspergillus* spp. (Photograph- i). Seeds collected from healthy plants were observed to be free from pathogens.



Photographs i. (a) Conidiaphone and conidia of *Aspergillus* spp., (b) Conidia of *Bipolaris oryzae*, (c) Conidia of *Pyricularia oryzae*, (d) Conidia of *Curvularia lunata*

General observation: After the seed was treated with the various fungicides a significant reduction in the pathogen population was observed and recorded from the fungicide treated seeds as compared to the untreated control.

(i) Laboratory studies-2020 and 2021:

a. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under laboratory conditions 2020.

The germination percent were examined, and seeds treated with Amistar Xtra 28 SC 3.7ml/L showed the lowest percent germination (61%), followed by Tridium 70 WG 4.3g/L (63%); while on the other hand, seed treated with Saaf 75 WP 5.6g/L recorded the highest percentage germination (91%), the untreated and healthy control recorded 80% mean percent germination (Table 2) under laboratory and pot culture during 2020.

Seed treatment under *in vitro* conditions at 14 DAS revealed seeds treated with Saaf at 5.6g/L produced seedlings with significantly longer shoots (5.04cm) as compared to other treatments, this was followed by the healthy control (4.67cm), Rodazim at 3.7ml/L (4.56cm), Saaf at 3.1g/L (4.34cm), Rodazim at 1.2ml/L (4.31cm), Antracol at 6.2g/L (4.28cm) and the untreated control (4.25cm). Treatment with Carbendazim was observed to have produced seedlings with the shortest shoots (2.99cm). Seedlings with the longest roots also came from seeds treated with Saaf at 5.6g/L (4.30cm), followed by those treated with Saaf at 3.1g/L (4.27cm) and Antracol at 6.2g/L (3.56cm). Treatments with Amistar Xtra at both rates 2.5ml and 3.7ml produced seedlings with significantly shorter roots as compared to all other treatments (1.68cm and 1.09cm, respectively). The seedling vigour index of rice seedlings evaluated under *in vitro* conditions indicated both treatments with Saaf at 5.6g/L and 3.1g/L produced seedlings with the highest vigour (849.9 and 731.9 respectively), this was followed by treatments of Antracol at 6.2g/L (627.00) and Rodazim at 3.7ml/L (614.80) Table 2). Seedlings with the lowest vigour index were from seeds treated with both rates of Tridium 2.5g and 3.7g/L (387.30 and 343.60, respectively), both treatments of Amistar Xtra 2.5ml and 3.7ml/L (374.80 and 231.00, respectively) and treatment with Carbendazim (367.10) (Table 2).

Data collected at 21 DAS indicated both treatments of Saaf i.e. at 5.6g/L and 3.1g/L produced seedlings with the longest shoots and roots (shoot: 5.09cm and 4.82cm, respectively, root: 3.99cm and 3.89cm, respectively) (Table 2); On the other hand both treatments with Amistar Xtra (2.5ml and 3.7ml/L) produced seedlings with the shortest shoot and root (shoot: 2.74cm and 2.21cm, respectively, root: 1.76cm and 1.15cm, respectively). Other treatments with relatively longer shoots were Rodazim at 1.2ml/L and 3.7ml/L (4.32 and 4.14cm, respectively), the healthy control (4.09cm), Antracol (3.80cm), the untreated control (3.79cm) and Glory 12.4g/L (3.67cm). Other treatments with significantly longer roots similar to that of treatments with Saaf were Manzate (3.56cm) and Antracol (3.33cm) (Table 2). A look at the SVI showed both treatments with Saaf (5.6g/L and 3.1g/L) produced seedlings with the higher vigour index (826.90 and 740.60, respectively) while treatments with Glory at 7.4g and Rodazim at 3.7ml/L followed close behind with SVI of 595.1 and 595.5, respectively. At the lower end of the spectrum with SVI of 337.80 and 204.80 were treatments with Amistar Xtra at rates of 2.5ml and 3.7ml/L, respectively (Table 2, Figure 1).

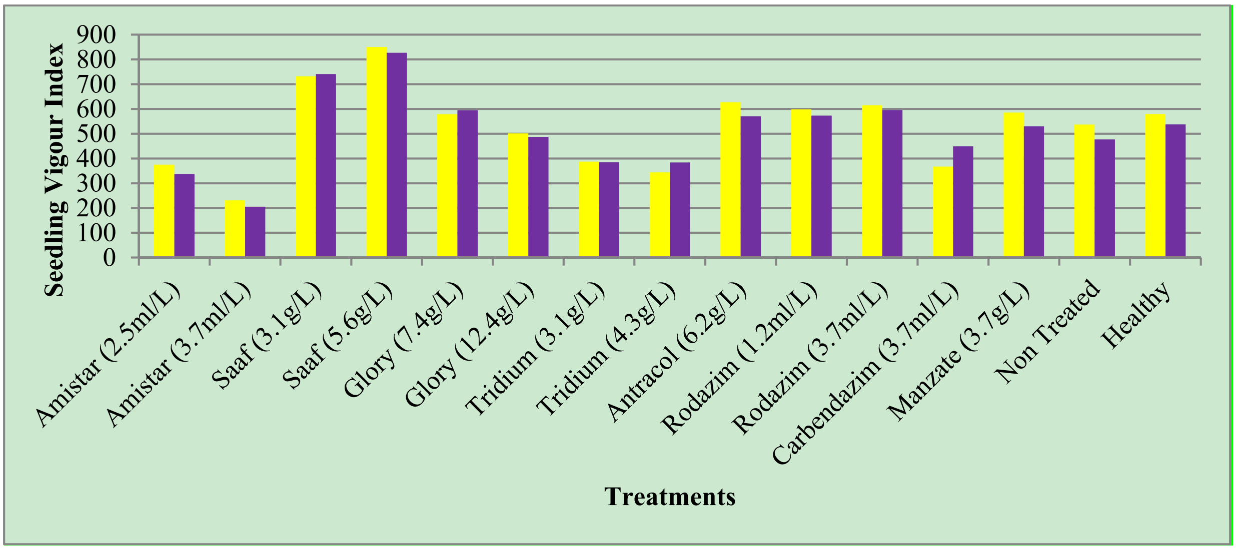


Figure 1. Seedling Vigour Index (SVI) at 14 and 21 days after treatment of seed treatment trial carried out under *in vitro* conditions in 2020

b. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under laboratory conditions- 2021.

After the evaluation of fungicides as seed treatment under *in vitro* conditions at 14 DAS, it was observed that seeds treated with Saaf at both 3.1g/L and 5.6g/L produced seedlings not with only the longest shoots (3.90 and 3.80cm, respectively) but also the longest roots (4.8 and 4.69cm respectively) and ultimately the highest seedling vigour (757.80 and 825.60, respectively) over all other treatments (Figure 2). Other treatments which performed exceptionally well were Manzate at 3.7g /L with shoot length of 3.40cm and Rodazim at 3.70ml/L with seedlings having average root lengths of 4.30cm and a seedling vigour index of 625.10 (Table 3, Figure 2).

Observations were then taken after 21 days of sowing which revealed that treatments with Saaf at both rates (3.1 and 5.6g/L) continued to outshine the others in shoot length (4.40 and 4.50 cm, respectively), root length (4.60 and 5.10cm respectively) and seedling vigour index 784.50 and 913.30, respectively. The healthy control was also observed to have significantly longer roots as compared to other treatments (4.80cm); while both treatments with Rodazim 1.2ml/L and 3.7ml/L recorded significantly higher seedling vigour index 685.90 and 689.00, respectively (Table 3, Figure 2).

Both treatments of Amistar Xtra (2.5 and 3.7ml/L) produced the shortest shoots 1.90 and 1.80cm, respectively and the lowest seedling vigour index 440.9.00 and 378.70, respectively (Table 3, Figure 2).

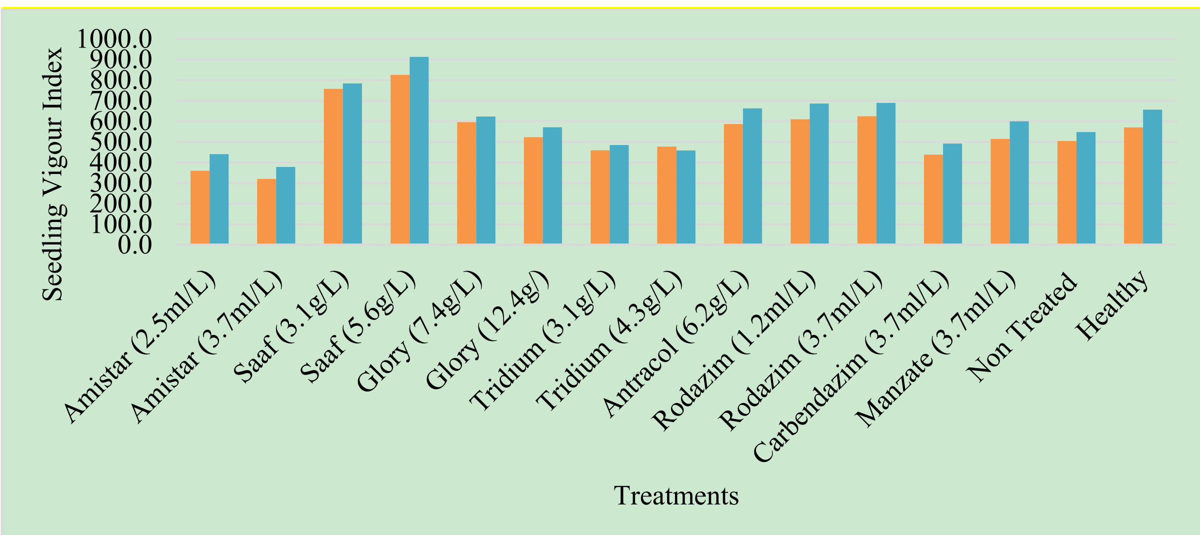


Figure 2. Seedling Vigour Index (SVI) at 14 and 21 days after treatment of seed treatment trial carried out under *in vitro* conditions in 2021

Table 2. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under laboratory condition 2020

Treatment	Product/ Lt	% Germination	14DAS		SVI	21 DAS		SVI
			Shoot length	Root length		Shoot length	Root length	
Amistar Xtra 28 SC	2.5ml	75	3.32 CD	1.68 FG	374.80 E	2.74 EF	1.76 HI	337.80 F
Amistar Xtra 28 SC	3.7ml	61	2.69 D	1.093 G	231.00 F	2.21 F	1.15 I	204.80 G
Saaf 75 WP	3.1g	85	4.34 AB	4.27 A	731.90 B	4.82 AB	3.89 AB	740.60 A
Saaf 75 WP	5.6g	91	5.04 A	4.30 A	849.90 A	5.09 A	3.99 A	826.90 A
Glory 75 WG	7.4g	87	3.15 CD	3.50 B	578.60 CD	3.67 CD	3.17 CDE	595.10 B
Glory 75 WG	12.4g	83	2.73 D	3.31 BC	501.10 D	2.67 EF	3.20 BCDE	487.20 CDE
Tridium 70 WG	3.1g	74	2.76 D	2.48 DE	387.30 E	2.80 EF	2.41 FGH	385.30 EF
Tridium 70 WG	4.3g	63	3.14 D	2.32 EF	343.60 E	3.17 DE	2.92 CDEF	383.90 EF
Antracol 70WP	6.2g	80	4.28 AB	3.56 AB	627.00 C	3.80 CD	3.33 ABCD	570.10 BC
Rodazim 50 SC	1.2ml	81	4.31 AB	3.08 BCD	598.30 CD	4.32 ABC	2.75 DEFG	573.20 BC
Rodazim 50 SC	3.7ml	85	4.56 AB	2.67 CDE	614.80 C	4.14 BC	2.87 CDEF	595.90 B
Carbendazim 50 SC	3.7ml	80	2.99 D	1.60 FG	367.10 E	3.56 CDE	2.05 GH	449.50 DEF
Manzate Pro Stick TM	3.7g	79	3.93 BC	3.48 B	585.40 CD	3.15 DE	3.56 ABC	529.90 BCD
Non Treated Control	-	80	4.25 AB	2.46 DE	537.00 CD	3.79 CD	2.17 GH	476.80 CDE
Healthy Control	-	80	4.67 AB	2.570CDE	579.40 CD	4.09 BC	2.63 EFG	537.60 BCD
Grand Mean			**3.74**	**2.82**	**527.14**	**3.60**	**2.79**	**512.96**
CV(%)			**12.58**	**15.89**	**11.60**	**13.76**	**14.79**	**12.15**

* = average of three replication

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher’s Least Significant Difference (LSD) procedures

Table 3. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under laboratory condition 2021

Treatment	Product/ Lt.	*% Germination	*14 DAS			*21 DAS		
			Shoot length (cm)	Root length (cm)	*SVI	Shoot length (cm)	Root length (cm)	*SVI
Amistar Xtra 28 SC	2.5ml	81.00	1.70 HI	2.80 GH	359.90 GH	1.90 FG	3.50 DE	440.90 HI
Amistar Xtra 28 SC	3.7ml	80.00	1.60 I	2.40 H	320.30 H	1.80 G	3.00 E	378.70 I
Saaf 75 WP	3.1g	87.00	3.90 A	4.80 AB	757.80 A	4.40 AB	4.60 AB	784.50 B
Saaf 75 WP	5.6g	95.00	3.80 AB	4.90 A	825.60A	4.50 A	5.10 A	913.30 A
Glory 75 WG	7.4g	89.00	3.10 BCDE	3.60 CDEFG	596.00 BC	3.60 ABC	3.40 DE	623.00 CDE
Glory 75 WG	12.4g	90.00	3.20 BCD	2.70 GH	523.20 BCDEF	3.40 CD	3.00 E	570.90 DEF
Tridium 70 WG	3.1g	88.00	2.30 FGH	2.90 FGH	459.30 EFG	2.60 DEFG	3.00 E	484.90 FGH
Tridium 70 WG	4.3g	79.00	2.90 CDEF	3.20 DEFGH	477.20 DEF	2.40 EFG	3.40 DE	458.70 GHI
Antracol 70WP	6.2g	82.00	3.20 BCD	3.90 BCDE	586.80 BCD	3.60 ABC	4.50 ABC	662.80 CD
Rodazim 50 SC	1.2ml	91.00	2.70 DEFG	3.90 BCD	610.30 BC	3.10 CDE	4.50 ABC	685.90 C
Rodazim 50 SC	3.7ml	88.00	2.80 CDEF	4.30 ABC	625.10 B	3.10 CDE	4.70 AB	689.00 C
Carbendazim 50 SC	3.7ml	83.00	2.30 FGH	3.00 EFGH	438.00 FG	2.30 EFG	3.60 CDE	491.60 FGH
Manzate Pro Stick TM	3.7ml	80.00	3.40 ABC	3.00 EFGH	514.40 BCDEF	3.50 BC	4.00 BCD	600.30 CDE
Non-Treated Control		85.00	2.10 GHI	3.8 CDEF	504.6 CDEF	2.5 EFG	4.00 BCD	548.00 EFD
Healthy Control		87.00	2.50 EFG	4.0 ABCD	570.7 BCDE	2.8 CDEF	4.80 AB	656.60 CD
Grand Mean			**2.77**	**3.5556**	**544.62**	**3.0356**	**3.92**	**599.27**
CV			**12.91**	**15.54**	**12.46**	**17.60**	**13.31**	**9.36**

[*] = average of four replication

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedures.

(II) POT CULTURES-2020 AND 2021:

a. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under pot condition- 2020

At 14 and 21 days after sowing (DAS) the root and shoot length of ten seedlings were measured and the seedling vigour index determined. Based on observations the average length of the longest shoot was 29.54cm at 14 DAS, this was recorded from seedlings treated with Rodazim at 3.7ml/L. Other treatments which produced relatively longer shoots were the non-treated control (27.77cm), Glory at 12.4g/L (27.70cm) and Saaf at 5.6g /L (27.24cm). Treatment with Amistar Xtra at 3.7ml/L. however produced the shortest shoots (20.91cm). The longest average root length on the other hand was obtained from seedlings treated with Rodazim at 1.2ml/L (7.17cm), followed by the non-treated control (6.13cm); while treatments with Tridium produced seedlings with the shortest average root length (3.72cm) (Table 4). A look at the seedling vigour index revealed treatment with Saaf at 5.6g /L produced seedlings with the highest seedling vigour index (2923.50), followed by treatments with Rodazim at 3.7ml/L (2946.70), Glory at 7.4g/L (2883.8) and Rodazim at 1.2ml/L (2747.3). Meanwhile seedlings with the lowest vigour index were those treated with Tridium at 4.3g/L (1960.2) and Amistar Xtra at 3.7ml/L. (1574.8) (Table 4, Figure 3).

Observations taken at 21 DAS showed similar trending results for few of the treatments. Seeds treated with Rodazim at 3.7ml/L. continued producing seedlings with the longest average shoots (34.05cm), followed by Glory at 12.4g/L (32.99cm), Rodazim at 1.2ml/L (32.74cm), the healthy control (32.29cm), Tridium at 3.1g/L (31.79cm) and Glory at 7.4g/L (31.27cm). Treatment with Amistar Xtra at 3.7ml/L again produced on average the seedlings with the shortest shoots (20.69cm). Seedlings with the longest average root length were again recorded from seeds treated with Rodazim at 1.2ml/L (10.42cm), followed by the healthy control (8.28cm), the non-treated control (7.95cm), and Carbendazim (7.93cm); while the shortest roots were obtained from treatment with Amistar Xtra at 3.7ml/L (4.85cm) (Table 4). A look at the seedling vigour index at 21 DAS revealed seedlings treated with Rodazim at 1.2ml/L had the highest SVI (3496.5), followed by Rodazim at 3.7ml/L (3399.5), Glory at 12.4g/L (3339.1) and also at 7.4g/L (3361.1), while treatment with Amistar Xtra at 3.7ml/L produced seedlings with the lowest vigour (1557.7) (Table 4, Figure 3).

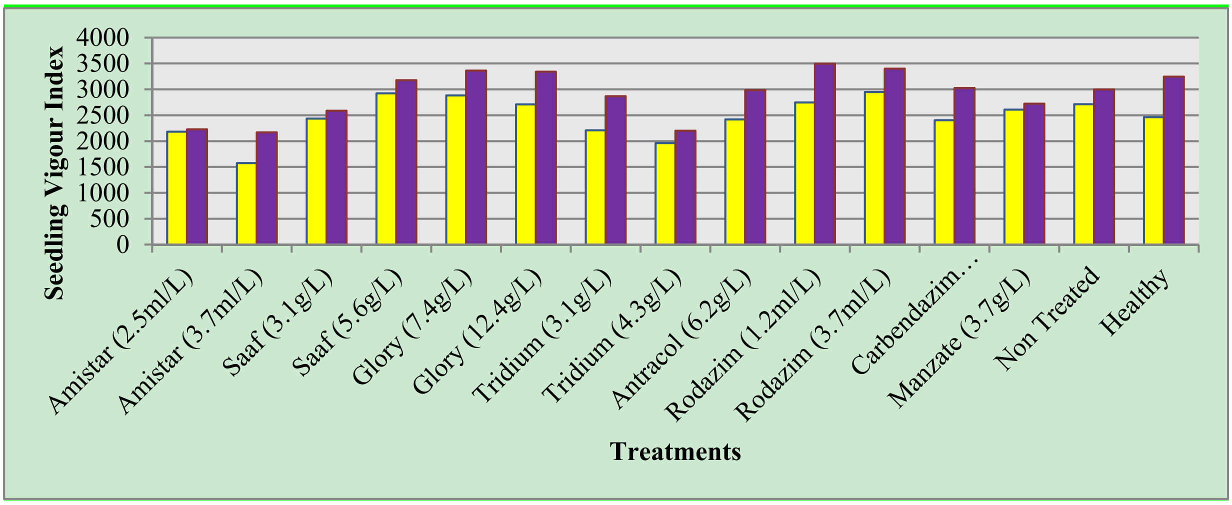


Figure 3. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under pot condition at 14 and 21 DAS during 2020

b. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under pot condition- 2021

Likewise, during 2021 the germination percent was recorded under laboratory and pot trial conditions, and the percentage germination ranges from 79% to 95%. Seeds treated with Tridium 70 WG 4.3g/L showed the lowest percent germination (79%), while on the other hand seed treated with Saaf 75 WP 5.6g/L recorded the highest percent germination (95%). The untreated and healthy control recorded 85% and 87% mean percent germination (Table 5). This gives an indication of the reliability of the germinating quality of seeds used within the laboratory and pot trial conducted in 2021.

Results of the effects of fungicide treated seeds on seedling growth and vigour revealed that seeds treated with Glory at 7.4g/L had the longest shoots (29.50cm) after fourteen days of sowing, followed by treatments with Glory at 12.4g/L (23.40cm). Seedlings with the shortest shoots were those coming from seeds treated with Amistar Xtra at 3.7ml/L (18.20cm), no significant difference in shoot length was observed among the other treatments evaluated (Table 5). Root length however was longest in seedlings whose seeds were treated with Rodazim at 1.2ml/L and shortest in those treated with Amistar Xtra 2.5 and 3.7ml/L, Tridium at 4.3g/L, Antracol and Manzate which were 3.70, 3.20, 3.50, 3.70 and 3.50cm, respectively. An overall look at seedling vigour index showed treatments with Glory at 7.4g/L had the highest seedling vigour 3010.90, followed by treatments with Saaf at 5.6g/L (2559.30) and Glory at 12.4g/L (2504.10); while the lowest seedling vigour came from seeds treated with Amistar Xtra at 3.7ml/L (1710.40) (Table 5, Figure 4).

Table 4. Effects of seed treatment using fungicides on germination and seedling vigour index, under pot condition-2020

Treatment	Product/ Lt	*% Germination	*14 DAS		*SVI	*21 DAS		*SVI
			Shoot length	Root length		Shoot length	Root length	
Amistar Xtra 28 SC	2.5ml	75	23.99 DE	5.11 ABC	2182.50 FG	23.89 BCD	5.82 BC	2228.10 DE
Amistar Xtra 28 SC	3.7ml	61	20.91 F	4.91 ABC	1574.80 H	20.69 D	4.85 C	1557.70 E
Saaf 75 WP	3.1g	85	22.99 EF	5.65 ABC	2433.90 DEF	23.45 CD	6.99 BC	2587.30 CD
Saaf 75 WP	5.6g	91	27.24 ABC	4.89 ABC	2923.50 A	28.66 ABC	6.26 BC	3177.90 ABC
Glory 75 WG	7.4g	87	27.37 ABC	5.78 ABC	2883.80 AB	31.27 AB	7.36 BC	3361.10 AB
Glory 75 WG	12.4g	83	27.70 AB	4.93 ABC	2708.80 ABCD	32.99 A	7.24 BC	3339.10 AB
Tridium 70 WG	3.1g	74	26.12 BCD	3.72 C	2208.20 FG	31.79 A	6.95 BC	2866.50 ABCD
Tridium 70 WG	4.3g	63	26.69 ABCD	4.43 BC	1960.20 G	29.21 ABC	5.68 BC	2198.20 DE
Antracol 70WP	6.2g	80	25.51 BCDE	4.73 BC	2419.00 DEF	30.01 ABC	7.31 BC	2985.40 ABC
Rodazim 50 SC	1.2ml	81	26.75 ABCD	7.17 A	2747.30 ABC	32.74 A	10.42 A	3496.50 A
Rodazim 50 SC	3.7ml	85	29.54 A	5.13 ABC	2946.70 A	34.05 A	5.95 BC	3399.50 AB
Carbendazim 50 SC	3.7ml	80	24.62 CDE	5.41 ABC	2402.20 EF	29.89 ABC	7.93 AB	3025.20 ABC
Manzate Pro Stick TM	3.7ml	79	27.41 ABC	5.62 ABC	2608.90 BCDE	27.16 ABCD	7.29 BC	2722.00 BCD
Non Treated Control		80	27.77 AB	6.13 AB	2712.0 ABCD	29.54 ABC	7.95 AB	2998.70 ABC
Healthy Control		80	25.46 BCDE	5.31 ABC	2461.6 CDEF	32.29 A	8.28 AB	3245.40 ABC
Grand Mean			**26.00**	**5.26**	**2478.20**	**29.18**	**7.09**	**2879.20**
CV			**6.69**	**26.13**	**7.26**	**15.22**	**24.65**	**14.75**

* = average of three replication

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedures.

At 21 DAS, it was clear that seeds treated with Rodazim at 3.7ml/L produced the longest average shoot length (30.90cm), this was followed by treatments with Manzate, (29.40cm), both rates of Glory 12.4 (28.80cm) and 7.4g/L (28.70cm) and Saaf at 5.6g/L (28.40cm). Those seeds treated with Saaf at 3.1g/L and Amistar Xtra 3.7ml/L recorded the shortest average shoot length (24.40 and 22.30cm, respectively). At 14 DAS, treatment with Rodazim at 1.2ml/L again produced the longest average root length (7.60cm), followed by the untreated control (6.30cm), Glory at 7.4g/ac (6.20cm) along with Manzate and Saaf at 3.1g/L both recording an average of 6.00cm root length after 21 days of sowing. The seedling vigour index at this stage was highest in both treatments of Rodazim 1.2ml/L with 3247.20 and 3.7ml/L with 3206.10, as well as treatments with Saaf 5.6g/L (3199.60), and Glory 7.4g/L (3107.30); while both treatments with Amistar Xtra yielded the lowest (2499.40 and 2206.70 respectively) (Table 5, Figure 4).

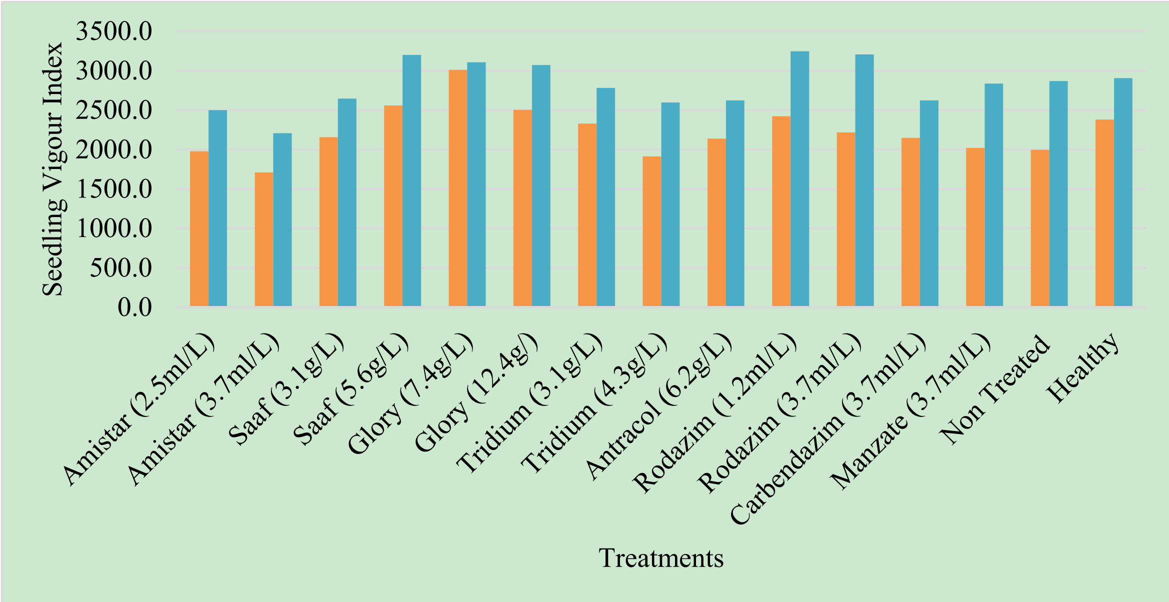


Figure 4. Effects of seed treatment using fungicides on Germination and Seedling Vigour Index, under pot condition at 14 and 21 DAS during 2021

Table 5. Effects of seed treatment using fungicides on germination and seedling vigour index, under pot condition-2021

Treatment	Product/ Lt.	*% Germination	*14 DAS		*SVI	*21 DAS		*SVI
			Shoot length (cm)	Root length (cm)		Shoot length (cm)	Root length (cm)	
Amistar Xtra 28 SC	2.5ml	81.00	20.80 BC	3.70 B	1977.80 CDE	25.40 DE	5.50 AB	2499.40 FG
Amistar Xtra 28 SC	3.7ml	80.00	18.20 C	3.20 B	1710.40 E	22.30 F	5.50 AB	2206.70 G
Saaf 75 WP	3.1g	87.00	20.50 BC	4.30 AB	2156.10 BCDE	24.40 EF	6.00 AB	2644.80 EF
Saaf 75 WP	5.6g	95.00	22.70 BC	4.30 AB	2559.30 AB	28.40 ABC	5.30 B	3199.60 AB
Glory 75 WG	7.4g	89.00	29.50 A	4.40 AB	3010.90 A	28.70 AB	6.20 AB	3107.30 ABC
Glory 75 WG	12.4g	90.00	23.40 B	4.40 AB	2504.10 B	28.80 AB	5.30 B	3070.80 ABCD
Tridium 70 WG	3.1g	88.00	22.30 BC	4.10 AB	2328.80 BCD	27.50 BCD	4.10 B	2781.10 DEF
Tridium 70 WG	4.3g	79.00	20.70 BC	3.50 B	1912.30 DE	28.10 BC	4.80 B	2597.50 EF
Antracol 70WP	6.2g	82.00	22.40 BC	3.70 B	2138.00 BCDE	26.90 BCDE	5.10 B	2624.30 EF
Rodazim 50 SC	1.2ml	91.00	21.30 BC	5.30 A	2422.40 BC	28.20 BC	7.60 A	3247.20 A
Rodazim 50 SC	3.7ml	88.00	21.40 BC	3.80 AB	2218.20 BCD	30.90 A	5.50 AB	3206.10 AB
Carbendazim 50 SC	3.7ml	83.00	22.00 BC	3.90 AB	2147.50 BCDE	25.90 CDE	5.70 AB	2623.70 EF
Manzate Pro Stick TM	3.7g	80.00	21.80 BC	3.50 B	2020.80 CDE	29.40 AB	6.00 AB	2835.50 CDE
Non-Treated Control	-	85.00	19.60 BC	3.90 AB	1995.50 CDE	27.50 BCD	6.30 AB	2868.50 CDE
Healthy Control	-	87.00	22.70 BC	4.70 AB	2379.20 BCD	27.70 BCD	5.70 AB	2904.90 CDE
Grand Mean			**21.95**	**4.03**	**2232.10**	**27.35**	**5.63**	**2827.8**
CV(%)			**14.09**	**23.09**	**12.92**	**5.64**	**23.90**	**6.82**

* = average of four replication; DAS= Days after sown;

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedures.

(III) FIELD STUDIES-2020 AND 2021:

(a) ***Effects of seed treatment using fungicides on growth parameters, yield parameters and overall grain yields-2020.***

Seeds treated with three fungicides *viz.* Rodazim, Manzate and Glory were found to be promising during studies in 2019 and therefore were selected for evaluation under field conditions in 2020 against an untreated control. There was however no significant difference in the panicle length, filled grains per panicle, 1,000-grain weight nor plant height among the various treatments (Table 6).

Table 6. Effects of seed treatment using fungicides on growth and yield parameters during 2020

Treatment/ Lt.	*Growth parameters		*Yield parameters			
	Plant Height (cm)	Tillers/m^2	Panicle Length (cm)	Filled Grains/ panicle	Unfilled Grains / panicle	1000 Grain Weight (g)
Rodazim @3.7ml/L	112.65 A	320.00 BC	25.65 A	169.00 A	53.00 BC	26.65 A
Manzate Pro Stick@ 3.7g/L	107.08 A	304.00 C	24.58 A	153.00 A	58.00 B	25.68 A
Glory @7.4g/L	108.47 A	368.00 AB	23.94 A	143.00 A	41.00 C	26.03 A
Control	107.33 A	396.00 A	25.10 A	146.00 A	75.00 A	27.00 A
Grand Mean	**108.88**	**348.00**	**24.82**	**153.00**	**57.00**	**26.33**
SEm ±	**2.63**	**6.94**	**0.77**	**195.82**	**67.12**	**0.93**
CD (P = 0.05)	**5.95**	**15.69**	**1.75**	**442.98**	**151.84**	**2.10**
CV	**3.42**	**11.32**	**4.40**	**18.13**	**16.74**	**5.00**

* = average of four replication

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedures.

With regards to the number of unfilled grains per panicle, treatment with Glory was observed to have produced significantly less unfilled grains; while the untreated control produced the most followed by Manzate, then Rodazim. The untreated control recorded the largest number of tillers/m^2, followed by Glory, Rodazim, then Manzate Pro Stick (Table 6). Observations also indicated seeds treated with Rodazim and Manzate had the highest average plot grain yield as compared to those treated with Glory and the untreated control (Table 7).

Table 7. Effects of seed treatment using fungicides on grain yield during 2020

Treatment/ Lt.	Plot Yield	
	Kg/ha	Bags/ac
Rodazim @3.7ml/L	5188.50 A	33.73 A
Manzate Pro Stick@ 3.7g/L	5001.40 A	32.52 A
Glory @7.4g/L	3834.30 AB	24.93 AB
Control	3546.10 B	23.25 B
Grand Mean	**4392.60**	**28.605**
SEm ±	**624.22**	**4.084**
CD (P = 0.05)	**1412.10**	**9.23**
CV	**20.10**	**20.19**

* = average of four replication

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedures.

(b) ***Effects of seed treatment using fungicides on growth parameters, yield parameters and overall grain yields-2021.***

Seeds treated with the said three fungicides *viz.* Rodazim, Manzate and Glory were re-evaluated under field conditions 2021 and compared against an untreated control.

While there was no significant difference observed among treatments for the overall yield of the spring crop 2021, nor any of the major yield attributing characters such as tillers/m^2, 1000 grain weight and unfilled grains/ panicle, some difference was observed in the length of panicles, filled grains/ panicle and plant height (Table 8). Treatments with Manzate were observed to not only produce the longest panicles (25.48cm), followed by Glory (24.33cm) but it also the largest number of filled grains/ panicle (160.00) there was no significant difference among other treatments. Plant height ranged from 100.82cm in the untreated control to 95.40cm in treatments with Rodazim (Table 8).

In the autumn crop of 2021, data collected revealed no significant difference among any of the treatments for grain yield, number of tillers/m2, plant height, the weight per 1000 grain nor the number of unfilled grains per panicle, what did show signs of variation however was the length of panicles and the number of filled grains per panicle (Table 9).

Table 8. Effects of seed treatment using fungicides on growth and yield parameters during spring crop 2021

Treatment	***Panicle Length (cm)**	***Filled grains/ Panicle**	***Unfilled grains/ Panicle**	***1000 grain weight (g)**	***Plant Height (cm)**	***Tillers/ m^2**	***Kg/ha**	***Bags/ac**
Rodazim @3.7ml/L	23.23 B	121.00 B	14.00 A	23.33 A	95.40 B	263.00 A	5290.40 A	34.38 A
Manzate Pro Stick@ 3.7g/L	25.48 A	160.00 A	17.00 A	23.00 A	95.80 B	260.00 A	5311.20 A	34.53 A
Glory @7.4g/L	24.33 AB	131.00 B	13.00 A	25.00 A	97.05 AB	273.00 A	5095.80 A	33.13 A
Control	23.60 B	127.00 B	13.00 A	23.78 A	100.82 A	262.00 A	4387.20 A	28.53 A
Grand mean	24.16	135.00	14.00	23.738	97.27	265.00	5021.20	32.64
SEM	0.60	7.78	2.1950	0.93	1.94	21.93	472.91	3.088
CD (P=0.05)	1.36	17.60	4.9653	2.11	4.40	49.62	1069.80	6.99
CV (%)	3.52	8.17	21.72	5.56	2.83	11.73	13.32	13.38

* = average of four replication

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedures.

Table 9. Effects of seed treatment using fungicides on growth and yield parameters during autumn crop 2021

Treatment	* Panicle Length (cm)	* Filled grains/ Panicle	* Unfilled grains/ Panicle	* 1000 grain weight	* Plant Height	* Tillers/ m^2	* Kg/ha	* Bags/ac
Rodazim @3.7ml/L	27.54 A	164.00 AB	21.00 A	23.93 A	111.83 A	139.00 A	5204.60 A	33.83 A
Manzate Pro Stick@ 3.7g/L	26.85 AB	174.00 A	26.00 A	23.75 A	108.70 A	153.00 A	5727.00 A	37.20 A
Glory @7.4g/L	25.55 B	143.00 BC	21.00 A	26.10 A	112.25 A	168.00 A	4844.20 A	31.50 A
Control	26.18 AB	121.00 C	22.00 A	23.25 A	106.03 A	156.00 A	4854.90 A	31.55 A
Grand mean	26.53	150.00	22.00	24.26	109.70	154.00	5157.70	33.52
SEM	0.82	11.83	3.70	2.63	4.10	18.32	747.12	4.852
CD (P=0.05)	1.78	25.78	8.05	5.74	8.95	39.92	1627.80	10.57
CV (%)	4.35	11.13	23.42	15.35	5.29	16.82	20.49	20.47

* = average of four replication

Means values in columns followed by same letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedures.

Seeds treated using Rodazim produced significantly longer panicles (27.54cm) as compared to that of other treatments, second in the lead was Manzate at 26.85cm, the untreated control (26.18cm) and finally Glory with (25.55cm). With regards to the number of filled grains per panicle, treatment with Manzate produced the largest number of grains (174.00) which was significantly greater than that produced by Rodazim (164.00), Glory (143.00) and the untreated control (121.00) (Table 9).

4. Discussion

Germination and seedling vigour are important attributes which determines the quality of seeds and predicts the faith of any seed lot under biotic and abiotic stress (Mahal, 2016). The results of the germination test during 2020 showed variability among treatments, percentage germination ranged from 61% in seeds treated with Amistar Xtra 28 SC 3.7ml/L to 91% in seeds treated with Saaf 75 WP 5.6g/L. While in 2021 the percentage germination ranged from 79% in seeds treated with Tridium 70 WG 4.3g/L to 95% in seed treated with Saaf 75 WP 5.6g/L. In 2020 the healthy pre-sterilized control and untreated control recorded similar percent germination (80%), while in the 2021 trials the healthy pre-sterilized control demonstrated slightly higher percentage germination (87%) over that of the untreated control (85%). The higher germination rate indicates that the fungicides in this trial helped protect the seedling against adverse conditions caused by unwanted pathogenic microorganism that might have negatively affected the seeds' ability to germinate. The research conducted by Taye et al., (2013) agreed with the findings of this research. The authors observed and reported an increase in germination values of treated seeds compared to untreated control which they concluded may be specific responses of *Z. mays* to the treatment fungicides. Additionally, Naher et al., (2016) found when seeds treated with the chemical fungicide Vitavax 200 (0.25%) recorded higher percent germination compared to the untreated seeds due to the presence of several pathogenic microorganisms observed on the rice seeds before treatment was applied when the dry inspection and blotter test was carried out. Additionally, Attri et al., (2018) also reported that pre-sowing seed treatments have proven to be an effective technique in enhancing seed germination, germination rate, uniformity, and seedlings growth under both normal and stress conditions. Likewise, Akgul et al., (2011) found that fungicides applied as seed treatment decreased the germination disorders caused by some fungal agents (*Aspergillus* spp., *Rhizopus* sp., *Penicillium* spp.) in and/or on the surface of seeds significantly and treatments provided an increase in germination ratio of seeds by 64-96%; and hence, agrees with the findings of this present research. This present research also observed and identified four dominant pathogens genera's (*Curvularia lunata, Bipolaris oryzae, Pyricularia oryzae* and *Aspergillus* spp.) from the rice seeds before seed treated and in the untreated control after treatment applied. The researchers Naher et al., (2016) made similar observation which agrees with the finding of this study. The researchers found seed borne fungi belonging to six genera [*Bipolaris oryzae, Alternaria padwickii, Fusarium moniliforme, Fusarium oxysporum, Curvularia lunata* and *Aspergillus* spp.] before the rice seeds were treated with the chemical Vitavax 200 and reported a significant reduction in the percent of these seed borne fungi after the seeds were treated with Vitavax 200 (0.25%). Similarly, Islam and Borthakur, (2012) also found species of *Aspergillus, Fusarium, Alternaria and Curvularia* to be the dominant genera when conduct paddy seed treatment trials. The similar observation was recorded and reported in this present study. Also, another researcher Shukl et al., (2008) found and reported that germination percent, plumule length, radicle length and seedling vigour index in case of lentils and *Terminalaria arjuna* showed better performance for all these attributes when seed treated with chemical before sown. Ibiam et al., (2008) also reported that rice seed treated with fungicides (such as Bavistin, Benlate, Fernasan-D, Apron Plus 50 DS and Dithane–M45) showed that all the fungicides significantly inhibited the seed-borne fungi associated with the rice seeds and enhance germination. Similarly, Butts et al., (2011) found chemical fungicides namely antracal, topsin, and mencozeb markedly suppressed the growth of *Helminthosporium sp. And Curvularia* sp. By more than 50%.

The beneficial impact of seed treatment was also recorded for shoot and root length. Results of the *in vitro* studies carried out in 2020 and 2021 saw treatments with Saaf at 5.6g/L produced seedlings with significantly longer shoots (5.04cm) and longest roots (4.30cm) as compared to other treatments and the untreated control at 14 DAS. Likewise, a similar trend was observed for data collected at 21 DAS. Both treatments of Saaf i.e. at 5.6g/L and 3.1g/L produced seedlings with the longest shoots (5.09cm and 4.82cm, respectively) and roots (3.99cm and 3.89cm, respectively). A similar trend was found in the 2021 trial where both rates of Saaf continue to show higher shoot and root length compared to the other treatments and the untreated control. Other treatments with noticeably longer shoots and roots were that of Rodazim at 3.7ml/L, Glory at 12.4g/L, Manzate Pro Stick TM at 3.7g/L and the healthy control. Similar results were reported when adivistin and haydazim 50 WP (carbendazim) at the rate of 0.4% were applied as seed-treating fungicides for the control of rice blast disease (Latif et al., 2011). The healthy control was observed to rank high among the other treatments while the untreated control had the shorter shoots and roots during the evaluation in 2020 and 2021.

The seedling vigour index of rice seedlings evaluated under *in vitro* conditions in 2020 and 2021 indicated treatments with Saaf (at 5.6g/L and 3.1g/L) produced seedlings with the highest vigour index, followed by seed treated with Antracol at 6.2g/L. Glory 75 WG 7.4g/L, Manzate Pro Stick TM at 3.7g/L and Rodazim at 3.7ml/L. Likewise, under pot culture condition a look at the SVI showed similar results where treatment with Saaf (5.6g/L) produced seedlings with the higher vigour index, while treatments with Glory (7.4g and 12.4g/L) and Rodazim (1.2ml/L and 3.7ml/L) followed close behind. Similar results were recorded by Shaid et al., (2011) who reported that seeds of chickpea treated with Vitavax were found superior for laboratory germination, root and shoot length, weight of seedling and seedling vigour index. Also, the vigour of rice seedlings increased when the seeds were treated compared to that of seedlings from the untreated seeds, along with increased in the germination capacity ranging from 5 to 13% for treated seeds was reported by Nguefack et al., (2008); and Filippi and Prabhu (1997) reported seed treatment with pyroquilon fungicide suppressed leaf blast until 62 and 47 days after seeding. It has therefore been determined that the use of pre-sowing fungicides as seed treatment is necessary with the objective of not only limiting or controlling the attack of pathogen but to enhance good plant stand at the beginning, particularly in cases where healthy seeds are unavailable to farmers; farmers can use seed treatment. All of the fungicides evaluated were observed to have a positive effect on germination, root and shoot length as compared to that of the unhealthy control.

5. Conclusion

It can be concluded from this research work that farmers should employ seed treatment prior to sowing if do not have access to clean, certified disease free seed paddy and have to use seed paddy that may be questionable or known to be harvest from fields that had symptoms of some of known major rice diseases, such as Blast, Brown spot, Sheath blight, Sheath rot or Grain discolouration, symptoms. It is recommended to use the paddy seed treatment [Saaf (5.6g/L), Manzate Pro Stick TM (3.7 g/L), Glory at (12.4g/L), Rodazim (3.7ml/L)] found to be effective as a method of controlling seed borne pathogens, enhancing germination, seedling vigour and ultimately improving productivity.

6. Recommendation

To draw a sound recommendation further investigation on the effects of pre sowing fungicidal seed treatment on the farmers' field and management conditions is warranted. The findings also underscore the need for a comparative study of germination and seedling growth of different varieties in response to a particular fungicide.

Author's Contributions

First author Rajendra Persaud design and carried out the experiments, analyzed the data and drafted the manuscript. All other authors provide technical advice, assisted with editing, moral support, read and agree with the content of the manuscript.

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Disclosure Statement

There is no potential conflict of interest to declare.

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