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EFFECTS OF PLANT EXTRACTS, BAU-BIOFUNGICIDE AND FUNGICIDES ON QUALITY AND HEALTH OF SEED

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Abstract

Extracts of garlic (*Allium sativum*) and neem (*Azadirachta indica*); BAU-Biofungicide (a *Trichoderma* based preparation) and Propiconazole and Carbendazim were sprayed for producing quality and health of seeds of rice cv. BR11. The highest increase (14.39%) in seed germination (%) was recorded over control when seeds were treated with BAU-Biofungicide (2 and 3%). BAU-Biofungicide (3%) resulted (45.75%) higher increase in vigour index over control. Most of the seed borne pathogens were inhibited by BAU-Biofungcde and Potent sprayed plot. *Trichoderma harzianum* was abundantly persistent in the harvested seeds of sprayed plot with BAU-Biofungicide as observed with incubated seeds.

Introduction

In Bangladesh, out of 32 diseases on rice 10 have the potentiality to cause economic damage to the crop (Haq *et al*. 2008). Fakir (2004) stated that roughly 10% loss of production of rice may be incurred annually due to seed borne diseases in Bangladesh and according to this estimate, 2.5 million tons of rice worth Tk. 30,000 millions are lost annually in Bangladesh. Good quality seeds possess major characteristics such as high yielding potentiality, viability, purity, free from varietals mixtures and being healthy i.e. free from infection by pathogens or having maximum acceptable tolerance limit of infection by a given pathogen in a given seed lot (Fakir and Mia 2004). Poor quality of rice seeds harbours many pathogens like fungi, bacteria and nematode (Mew and Gonzales 2002).

In Bangladesh, 27 of 43 diseases known to occur on rice are seed-borne (Fakir 2004). Seed borne diseases caused by fungi such as brown spot (*Bipolaris oryzae*), blast (*Pyricularia grisea*)**,** sheath rot (*Sarocladium oryzae*), seed rot and seedling blight (*Bipolaris oryzae*, *Sclerotium rolfsii* and *Fusarium* spp.) and grain spots (*Bipolaris oryzae***,** *Curvularia lunata***,** *Nigrospora oryzae***,** *Phoma glumarum* and *Cladosporium* sp.); by bacteria such as bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas oryzae* pv**.** *oryzicola*); and by nematode like white tip (*Aphelenochoides besseyi*) are harmful to rice seed health for inflicting diseases in seed bed as well as in field (Fakir 2004). The present study has been designed to control major rice seed borne diseases using plant extracts and biocontrol agents as an alternate means in order to avoid environmental pollution. Treating of seeds with BAU-Biofungicide resulted strong barrier on seed surface to protect seed from seed borne pathogens as well as it protects seed from soil borne pathogens when treated seeds are sown in field or in seed bed (Hossain 2011).

Materials and Methods

The experiment was conducted during two Aman seasons in the field Laboratory of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh. The field was fertilized as per recommendation of Bangladesh Rice Research Institute, Gazipur (BRRI 2004). The experiment was conducted with RCBD having three replications. The individual plot

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size was 5.0 m × 2.0 m (10 m^2). Block to block and plot to plot distances were 2.0 m and 1.5 m, respectively. Thirty four days old seedlings were uprooted from the seed bed and three seedlings per hill were transplanted in the field on August 4, 2011 and 2012. Hill to hill and row to row distances were 15 cm and 25 cm, respectively. The spray schedule was started just after the commencement of disease symptom and three sprays were made at 15 days interval.

Healthy leaves of neem and garlic cloves were collected and washed thoroughly under running tap water followed by sterile distilled water. The extracts were prepared by homogenizing 5 g of plant sample in 50 ml of sterile distilled water using a blender and the extracts were then prepared at 1 and 2% concentration by dilution with water and kept in conical flasks separately before use. BAU-Biofungicide is a *Trichoderma* based preparation (Hossain 2011). BAU-Biofungicide @ 2 and 3%, Bavistin DF (Carbendazim) and Potent 250 EC (Propiconazole) were also used both with @ 0.1 and 0.05% concentration.

Three hundred harvested seeds of rice cv. BR11 of control plot were treated with each plant extracts, separately over time by weight basis @ 1 and 2% and also treated with BAU-Biofungicide @ 2 and 3% and both with Bavistin and Potent @ 0.1, 0.05% of seed weight. The experiment was conducted in the nethouse of the Seed Pathology Centre, BAU, Mymensingh. Sand was collected from Brahmaputra river, Mymensingh. The collected sand was sterilized with formalin (40%) at the rate of 5 ml formalin diluted with 20 ml of water for 4 kg sand (Dashgupta 1988). The formalin treated soil was covered with polythene sheet for 48 hrs and then exposed for 48 hrs for aeration before setting experiment. The plastic trays (12″ × 8″) were filled with sand. The experiment was carried out in CRD with three replications. Three hundred seeds of each treatment were sown in plastic trays (100 seeds/tray) maintaining equal distances among the seeds. Plants were watered for maintaining proper moisture. Randomly selected 10 seedlings were uprooted carefully from each tray and washed thoroughly with running tap water. Data were recorded for each treatment at 14 days after sowing on different parameters. Vigour index (VI) was computed using formula of Baki and Anderson (1973): Vigor index = (mean shoot length + mean root length) × % germination

To detect the seed health test and association of seed borne pathogens with seeds blotter method was followed (ISTA 1996). Three layers of blotting paper (Whatman filter No.1) soaked in water and were kept at the bottom of a 9.0 cm dia. plastic Petri dish and thereafter 25 seeds were kept on filter paper. Four hundred harvested seeds of each treatment were taken randomly from each sample. The Petri dishes containing seeds were incubated at 20 ± 2ºC under alternating cycles of 12 hrs near ultra violet light and darkness for 7 days. Incubated seeds were examined under stereo-binocular microscope to record the incidence of different seed borne fungi. Seed borne infection was recorded from incubated Petri dishes and expressed in percentage (Agarwwal *et al*. 1989). Recorded data on different parameters were analysed statistically using MSTAT-C computer program and treatment means were evaluated for significance using DMRT following Gomez and Gomez (1984).

Results and Discussion

Effect of seed treatment with plant extracts, BAU-Biofungicide, Bavistin and Potent were evaluated (Table 1). The highest (37.88%) increase of normal seedlings obtained from treated seeds was observed with BAU-Biofungicide (2 and 3%). The highest (70.96%) reduction of diseased seedling was found in Potent (0.05%) followed by BAU-Biofungicide 2% having 61.28% over control (Table 1). Maximum (90%) reduction was achieved with BAU-Biofungicide (3%) in germination failure. The highest 28.62% shoot length was appeared with BAU-Biofungicide (3%). Higher increase 26.47% of root length was found over control by BAU-Biofungicide (2%).

Table 1. Effect of seed treatments with extracts of garlic and neem; BAU-Biofungicide, Bavistin and Potent on germination (%) and vigour index at 14 days after sowing of rice seeds of cv BR11 following Tray method.

Treatment (dose)	Germination (%)	Normal seedling (%)	Abnormal seedling (%)	Diseased seedling (%)	Germin. failure (%)	Hard seed (%)	Shoot length (cm)	Root length (cm)	Shoot weight (mg)	Root weight (mg)	Vigour index
Garlic (1%)	94.00a (+9.72)	84.00ab (+27.27)	3.67d (–60.66)	6.33bc (–38.72)	2.67cd (–73.3)	3.33b (–41.27)	9.97cd (+6.06)	12.43bcd (+12.29)	35.00cd (+16.67)	29.00cde (+11.54)	2103.48bc (+20.04)
Garlic (2%)	95.00a (+10.89)	86.00ab (+30.30)	3.33d (–64.31)	5.67cd (–45.11)	2.00cd (–80.00)	3.00bc (–47.09)	10.33cd (+9.89)	12.93abc (+16.80)	37.00abc (+23.33)	31.00bcd (+19.23)	2208.00b (+26.01)
Neem (1%)	94.00a (+9.72)	83.00ab (+25.76)	4.33d (–53.59)	6.67bc (–35.43)	2.67cd (–73.30)	3.33b (–41.27)	10.60cd (+12.77)	12.68abc (+14.54)	38.00abc (+26.67)	31.67bcd (+21.81)	2189.44b (+24.95)
Neem (2%)	94.00a (+9.72)	85.00ab (+28.79)	4.00d (–57.13)	5.00de (–51.60)	3.00c (–70.00)	3.00bc (–47.09)	10.59cd (+12.66)	13.17abc (+18.97)	38.67abc (+28.90)	32.33bc (+24.35)	2233.58b (+27.47)
BAU-Biofungicide (2%)	98.00a (+14.39)	91.00a (+37.88)	3.00d (–67.85)	4.00f (–61.28)	1.00d (–90.00)	1.00d (–82.36)	11.98ab (+27.45)	14.00a (+26.47)	39.00a (+30.00)	36.00a (+38.46)	2548.43a (+45.43)
BAU-Biofungicide (3%)	98.00a (+14.39)	91.00a (+37.88)	3.00d (–67.85)	4.00f (–61.28)	1.00d (–90.00)	1.00d (–82.36)	12.09a (+28.62)	13.97a (+26.20)	40.00a (+33.33)	35.67a (+37.19)	2554.03a (+45.75)
Bavistin DF (0.1%)	93.00a (+8.56)	83.00bac (+25.76)	4.00d (–57.13)	6.00bc (-41.92)	3.33b (–66.70)	3.67b (–35.27)	11.28abc (+20.00)	13.72ab (+23.94)	40.00a (+33.33)	36.00a (+38.46)	2325.25ab (+32.70)
Bavistin DF (0.05%)	92.00a (+7.39)	79.00b (+19.70)	6.00c (–35.69)	7.00b (–32.24)	4.33a (–56.70)	3.67b (–35.27)	10.75abcd (+14.36)	12.31bcd (+11.20)	36.00bcd (+20.00)	33.67ab (+29.50)	2124.71b (+21.25)
Potent 250 EC (0.1%)	96.00a (+12.06)	64.67c (–2.02)	27.33a (–192.93)	4.00f (-61.28)	2.00cd (–80.00)	2.00cd (–64.73)	7.03e (–25.21)	12.02cd (–8.58)	33.00de (-10.00)	28.00de (+7.69)	1829.06cd (+4.38)
Potent 250 EC (0.05%)	98.00a (+14.39)	69.00c (+4.55)	26.00a (–178.67)	3.00f (–70.96)	1.00d (–90.00)	2.00d (–64.73)	6.65e (–29.25)	11.73cd (–5.96)	31.00e (-3.33)	31.00bcd (+19.23)	1802.08d (+2.84)
Control (water)	85.67b	66.00c	9.33b	10.33a	10.00a	5.67a	9.40d	11.07d	30.00e	26.00e	1752.30d

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT. Data represent the means of three replications. Data in parentheses indicate per cent increased (+) and per cent decreased (–) over control. DAS = Days after sowing.

Maximum (14.39%) seed germination was increased when seeds were treated with BAU-Biofungicide (3%) over control, while BAU-Biofungicide (3%) resulted 45.75% higher increase in vigour index over control followed by Bavistin (0.1%) having 32.70%. BAU-Biofungicide and Bavistin (0.1%) also resulted the highest shoot weight (40 mg) and root weight (36.00 mg) indicating 33.33 and 38.46% higher increase in shoot and root weight, respectively over control. The results are supported by Ora *et al*. (2011) and Hossain (2012). Hossain (2012) reported that 28.25, 18.31 and 17.79% vigour index were increased over control when wheat seeds of Kanchon variety was treated with BAU-Biofungicide (2.5%), Bavistin (0.1%) and garlic (1%), respectively at 10 days after sowing. He also observed that 24.09% shoot weight and 30.47% root weight were increased with BAU-Biofungicide (2.5%), while Bavistin showed 18.87% shoot weight and root weight 17.42%. Mahmud and Hossain (2013) evaluated the efficacy of BAU-Biofungicide, garlic. neem, Bavistin and Potent of harvested seeds of rice cv. BR11. BAU-Biofungicide (3%) showed the highest germination (98.00%) and (93.00%) normal seedling. Ora *et al*. (2011) showed better performance in terms of lowest pathogenic incidence, rotten seed, dead seed, seed germination and seedling vigour index.

Plant extracts, BAU-Biofungicide and fungicides on health status of rice seeds were evaluated by standard blotter incubation test and the seeds were found to be associated with 10 different seed borne fungi belonging to nine genera *viz., Alternaria padwickii, Aspergillus flavus, Bipolaris oryzae, Curvularia lunata, Fusarium moniliforme, Fusarium oxysporum, Nigrospora oryzae, Penicillium spp., Sarocladium oryzae* and *Trichoderma harzianum* (Tables 2 and 3). The highest 90% reduction of seed-borne infection of *B. oryzae* was obtained in harvested seeds by spraying plots with BAU-Biofungicide 3% over control followed by Potent (0.1%) having 63.64% reduction. Maximum 40% reduction of seed-borne infection of *F. oxysporum* was found in BAU-Biofungicide 2% followed by 37.50% reduction with Potent (0.1%) over control. The highest (62.50%) reduction of *F. moniliforme* was found with BAU-Biofungicide (2%) followed by BAU-Biofungicide (3%) having 56.25% reduction over control. Higher reduction (67.57%) of seed-borne infection of *C. lunata* was recorded in BAU-Biofungicide (2%) followed by BAU-Biofungicide (3%) and Potent (0.1%). Hundred per cent reduction of *A. flavus* was found with BAU-Biofungicide (2 and 3%) and neem leaf extract (2%) over control. Cent per cent reduction was observed with BAU-Biofungicide (2%) and neem (1 and 2%) in *N. oryzae*. Cent per cent reduction of seed-borne infection of *S. oryzae* was found with BAU-Biofungicide (2%) and Potent (0.1%), while garlic (1%) showed 80.00% reduction (Tables 2 and 3).

Gogoi and Ali (2005) evaluated the efficacy of *T. harzianum* against rice brown spot pathogen *(B. oryzae). T. harzianum* inhibited the growth of rice brown spot pathogen. Ahmed *et al.* (2002) tested 12 seed samples of rice that were infected by *B. oryzae*. He used Bavistin, Hinosan, Tilt 250 EC and Dithane M-45 and four plants extracts *viz.*, bishkatali, onion, garlic and neem against *B. oryzae*. Dithane M-45 was the best with 100% inhibition of mycelial growth at 0.3%. Neem and garlic were effective against *B. oryzae* at 1 : 1 dilution. Hossain and Srikant (2002) reported that *T. harzianum* inhibited the growth of *F. oxysporum* and later overgrew the test fungus. Nisa *et al.* (2011) also evaluated Carbendazim, Hexaconazol, Bitertanol, Myclobutanil, Mancozeb, Captan and Zineb and extracts of *Allium sativum, Allium cepa* and *Mentha arvensis* for their effect on the inhibition of mycelial growth and spore germination of *F. oxysporum.* Maximum inhibition in mycelial growth was observed in hexaconazol at 1000 ppm. Sagar *et al.* (2005) reported that *T. harzianum* was effective in reducing seed borne infection of *F. moniliforme* and increasing seedling vigour and seed germination. Sarhan and Shibly (2003) observed that *T. harzianum* inhibited the growth of *C. lunata* associated with rice seeds. Sarhan and Shibly (2003) also reported that *T. harzianum* was superior to other bio-control agents in terms of reduction of infection of *A. niger*. Sempere and Santamarina (2008) investigated the fungal growth in dual

Table 2. Effect of extracts of garlic and neem; BAU-Biofungicide, Bavistin and Potent on germination (%) and seed borne fungi of rice seeds of cv. BR11 following Blotter method during Aman season in 2011.

Treatment (dose)	Germination (%)	Germin. failure (%)	*Alternaria padwickii*	*Bipolaris oryzae*	*Curvularia lunata*	*Fusarium moniliforme*	*Fusarium oxysporum*	*Nigrospora oryzae*	*Sarocladium oryzae*	*Trichoderma harzianum*	*Aspergillus flavus*
Garlic (1%)	94.50abc (+5.88)	5.50cd (–48.84)	1.00c (–50.00)	4.00cd (–60.00)	5.00ef (–50.00)	7.00bc (–12.50)	15.50abc (–8.82)	2.00d (–71.43)	1.50ef (–66.67)	0.00c	1.00d (–84.62)
Garlic (2%)	95.00abc (+6.44)	5.00cd (–53.49)	0.00d (–100.00)	3.50d (–65.00)	6.00de (–40.00)	6.25c (–21.88)	16.25ab (–4.41)	5.00c (–28.57)	1.75ef (–61.11)	0.00c	2.00c (–69.23)
Neem (1%)	95.50abc (+7.00)	4.50d (–58.14)	2.00a (–0.00)	11.50ab (+15.00)	6.75d (–32.50)	8.50a (–6.25)	16.75ab (1.47)	0.00e (–100.00)	4.25ab (–5.56)	0.00c	1.00d (–84.62)
Neem (2%)	95.25abc (+6.72)	4.75cd (–55.81)	0.00d (–100.00)	11.00ab (+10.00)	5.50de (–45.00)	8.00ab (–0.00)	15.00bcd (–11.76)	0.00e (–100.00)	3.25cd (–27.78)	0.00c	0.00e (–100.00)
BAU-Biofungicide (2%)	98.00ab (+9.80)	2.00ef (–81.40)	1.00c (–50.00)	2.00e (–80.00)	4.50ef (–55.00)	3.00e (–62.50)	14.00cde (–17.65)	0.00e (–100.00)	1.50ef (–66.67)	4.00b	0.00e (–100.00)
BAU-Biofungicide (3%)	99.00a (+10.92)	1.00f (–90.70)	0.00d (–100.00)	1.00e (–90.00)	4.00f (–60.00)	3.50de (–56.25)	13.00e (–23.53)	1.50d (–78.57)	1.00f (–77.78)	5.00a	0.00e (–100.00)
Bavistin DF (0.1%)	94.00bc (+5.32)	6.00c (–44.19)	0.00d (–100.00)	10.50ab (–5.00)	14.50a (+45.00)	7.00bc (–12.50)	15.00bcd (–11.76)	2.50d (–64.29)	3.00d (–33.33)	0.00c	5.00b (–23.08)
Bavistin DF (0.05%)	92.00cd (+3.08)	8.00b (–25.58)	1.50b (–25.00)	11.75a (+17.50)	13.00b (+30.00)	7.25abc (–9.38)	15.50abc (–8.82)	5.50bc (–21.43)	3.75bc (–16.67)	0.00c	5.00b (–23.08)
Potent 250 EC (0.1%)	98.00ab (+9.80)	2.00ef (–81.40)	0.00d (–100.00)	5.00cd (–50.00)	5.00ef (–50.00)	4.00de (–50.00)	12.25e (–27.94)	5.75bc (–17.86)	0.00g (–100.00)	0.00c	2.00c (–69.23)
Potent 250 EC (0.05%)	97.00ab (+8.68)	3.00e (–72.09)	0.00d (–100.00)	5.50c (–45.00)	5.00ef (–50.00)	4.50d (–43.75)	13.50de (–20.59)	6.50ab (–7.14)	2.00e (55.56)	0.00c	2.50c (–61.54)
Control (water)	89.25d	10.75a	2.00a	10.00b	10.00c	8.00ab	17.00a	7.00a	4.50a	0.00c	6.50a

In a column, figures having same letter(s) do not differ significantly at 5 % level of significance by DMRT. Data represent the means of four replications. Data in parentheses indicate per cent increased (+) and per cent decreased (–) over control.

Table 3. Effect of extracts of garlic and neem; BAU-Biofungicide, Bavistin and Potent on germination (%) and seed borne fungi of rice seeds of cv. BR11 following Blotter method during Aman season in 2012.

Treatment (dose)	Germination (%)	Germin. failure (%)	*Alternaria padwickii*	*Bipolaris oryzae*	*Curvularia lunata*	*Fusarium moniliforme*	*Fusarium oxysporum*	*Nigrospora oryzae*	*Sarocladium oryzae*	*Trichoderma harzianum*	*Aspergillus flavus*
Garlic (1%)	93.50bcd (+ 4.76)	6.50c (–39.53)	0.00d (–100.00)	5.00c (–39.39)	3.75cd (–59.46)	5.00bc (–31.03)	16.00bc (–20.00)	1.00d (–76.47)	1.00c (–80.00)	0.00c	2.00c (–60.00)
Garlic (2%)	-	-	-	-	-	-	-	-	-	-	-
Neem (1%)	95.00abc (+ 6.44)	5.00d (–53.49)	2.00a (–0.00)	8.50b (+3.03)	5.00c (–45.95)	6.00ab (–17.24)	18.00ab (–10.00)	1.00d (–76.47)	2.75b (–45.00)	0.00c	1.00d (–80.00)
Neem (2%)	-	-	-	-	-	-	-	-	-	-	-
BAU-Biofungicide (2%)	99.00a (+ 10.92)	1.00f (–90.70)	0.00d (–100.00)	3.00d (–63.64)	3.00d (–67.57)	3.75cd (–48.28)	12.00d (–40.00)	1.00d (–76.47)	0.00d (–100.00)	6.50a	1.00d (–80.00)
BAU-Biofungicide (3%)	-	-	-	-	-	-	-	-	-	-	-
Bavistin DF (0.1%)	93.50bcd (+ 4.76)	6.50c (–39.53)	1.00c (–50.00)	9.00ab (+9.09)	10.75a (+16.22)	5.00bc (–31.03)	15.50c (–22.50)	1.50d (–64.71)	2.50b (–50.00)	0.00c	3.00b (–40.00)
Bavistin DF (0.05%)	91.50cd (+ 2.52)	8.50b (–20.93)	1.50b (–25.00)	10.50a (+27.2)	10.00a (+8.11)	6.25ab (–13.79)	15.50c (-22.5)	3.50bc (–17.65)	3.00b (–40.00)	2.75b	3.00b (–40.00)
Potent 250 EC (0.1%)	97.00ab (+ 8.68)	3.00e (–72.09)	1.00c (–50.00)	3.00d (–63.64)	3.75cd (–59.46)	3.75cd (–48.28)	12.50d (–37.50)	3.00c (–29.41)	0.00d (–100.00)	0.00c	1.00d (–80.00)
Potent 250 EC (0.05%)	95.50abc (+ 7.00)	4.50d (–58.14)	0.00d (–100.00)	4.00cd (–51.52)	4.00cd (–56.76)	3.25d (–55.17)	13.25d (–33.75)	3.75ab (–11.76)	0.00d (–100.00)	0.00c	1.50cd (–70.00)
Control (water)	89.25d	10.75a	2.00a	8.25b	9.25b	7.25a	20.00a	4.25a	5.00a	0.00c	5.00a

In a column, figures having same letter(s) do not differ significantly at 5% level of significance by DMRT. *Data represent the means of four replications.* Data in parentheses indicate per cent increased (+) and per cent decreased (–) over control. (–) = Not tested in 2012.

cultures where *T. harzianum* inhibited pathogenic growth before hyphal contact of *N. oryzae*. Kalaiselvi and Panneerselvam (2015) reported that *T. harzianum* was found to be most effective with 96% inhibition over control by dual culture. Jagannathan and Sivaprakasam (1996) tested the ability of neem (*A. indica*) derivatives (neem oil and neem seed kernel extract), leaf extract from *Vitex negundo, Acacia leucocephala* and *Polyanthia longifolia*, carbendazim and monocrtotophos to control sheath rot of rice compared with control. Under the present study no good effect was found with the extract of *A. indica* and *A. sativum* to protect the major seed borne pathogen though Razu and Hossain (2015) reported that garlic extract (5%) showed profound effect.

It was evident that prevalence of seed borne pathogens was reduced in Potent sprayed plot. Besides this, *T. harzianum* was abundantly persistent in harvested seeds of sprayed plot with BAU-Biofungicide as observed with incubated seeds and *T. harzianum* was associated with *B. oryzae* infected seeds and when kept for few days for incubation it overgrew *B. oryzae*. *T. harzianum* was associated even one year preservation of seeds of BAU-Biofungicide sprayed plot. BAU-Biofungicide showed superior effect over all other treatments in increasing germination of seeds, seedling growth and vigor index in treated seeds. Bavistin DF (0.1%) is the second most important chemical regarding as seed treating fungicide. So, BAU-Biofungicide (2%) can be used as foliar application for producing apparently healthy seeds as well as protecting seed borne pathogens and seed treating bioagent to develop plant stand and growth.

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