

## EFFICACY OF SEED TREATMENT IN IMPROVING SEED QUALITY IN RICE (*Oryza sativa* L.)

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### ABSTRACT

Eight seed lots were collected from respective breeders in 2003 through Division Seed Science and Technology, IARI, New Delhi were used in the present study. These eight seed lots were used to evaluate the efficacy of chemical seed treatment against *Bipolaris oryzae* and other seedborne fungi in vivo, and efficacy of chemical seed treatment against *Bipolaris oryzae* in vitro. Seeds were treated at @ 2.5 g/kg with Mancozeb, Thiram, Bavistin and Vitavax, and at 1ml/ kg with Neem oil, Palmarosa oil, Polykote and Seedkare Orange. Treated seeds were then packed in polythene bags 700-gauge and kept under ambient condition in Division of Seed Science and Technology, IARI, New Delhi from October 2003 to April 2004. The observations were recorded at 2-month interval upto 6 moths of storage on treated seeds to see the effect of pre-storage treatments on germination and incidence of *Bipolaris oryzae* and other seedborne fungi. Beside, persistence of different fungicides after storage was also investigated.

The results of the study showed that seeds treated with Vitavax, Thiram and Mancozeb maintained germination above MSCS ( $\geq 80\%$ ) after six months of storage, while other chemicals could not retain germination above MSCS. Palmarosa oil caused deterioration in germination below MSCS, it was due to its phytotoxic nature. Seed dyes viz. Polykote and Seedkare Orange did not harm to germination.

Most of the seedborne fungi viz. *Bipolaris oryzae*, *Alternaria padwickii*, *Curvularia lunata* and other seedborne fungi were eradicated by Vitavax, Thiram and Mancozeb. Whereas Bavistin was only effective slightly on *Alternaria padwickii* and *Curvularia lunata*, but similar to other chemicals did not affect on *Bipolaris oryzae* and other seedborne fungi. Field fungi decreased progressively, while storage fungi increased gradually during the increasing period of storage.

In the inhibition zone test, only Vitavax, Thiram and Mancozeb produced inhibition zone areas; they maintained considerable chemical residues on individual seed after six months of storage by 87.5, 82.5% and 65.9% as compared to the initial quantity of 62.5  $\mu\text{g}$  at seed treatment.

**Key words:** *Alternaria padwickii*, *Bipolaris oryzae*, *Curvularia lunata*, seedborne pathogen.

### INTRODUCTION

*Bipolaris oryzae* is a seedborne pathogen that causes brown spot of rice. This pathogen is prevalent in all rice growing countries of the world and most of the cultivars grown in the world are susceptible to it. Though it is considered as a minor disease, it is known to

cause considerable economic losses during normal years and at times like the great Bengal famine of 1942 (Padmanabhan et al. 1948).

The pathogen is known to cause damage at different stages like, storage, seed germination and seedling establishment, vegetable growth

and reproductive phase. As a result, productivity and quality of grains and seeds can be reduced considerably during production. As *Bipolaris oryzae* is a seedborne pathogen in nature, besides management of the disease through cultural practices, field chemical control, and chemical seed treatment has also been considered commonly in controlling *Bipolaris oryzae* and other seedborne fungi in rice (Dharam et al. 1970; Kauraw 1986; Rao et al. 1988; Ahmed et al. 2000; Parisi et al. 2001). Hence, in the present study fungicides and chemicals were used as seed treatment to evaluate their efficacy in controlling *Bipolaris oryzae* and other seedborne fungi to enhance the seed quality.

#### MATERIALS AND METHODS

Two rice hybrids along with their parental lines were used to conduct the study. They were PRH 10, P6-A, P6-B, PRR-78 and DRRH1, IR28025-A, IR28025-B, IR40750-R were collected from Haryana and Andhra Pradesh of India. Chemicals used for seed treatment viz. Mancozeb 50 WP, Thiram 75 SD, Bavistin 50 WP and Vitavax collected from market, Neem oil and Palmarosa oil from Division of Agricultural Chemicals, Indian Agricultural Research Institute (IARI), Polymer Polykote and seed dyer Seedkare Orange from chemical company.

These seed lots were divided into 9 sub-lots of 60 grams each. Eight sub-lots were treated with following chemicals, while the 9<sup>th</sup> sub-lot was maintained as untreated control.

No.	Chemicals	Dose
1	Mancozeb (DM-45 ) 50 WP	2.5 g/ kg
2	Thiram 75 SD	2.5 g/ kg
3	Bavistin (Carbendazim) 50 WP	2.5 g/ kg
4	Vitavax	2.5 g/ kg
5	Neem oil	1 ml/ kg
6	Palmarosa oil	1 ml/ kg
7	Polykote	1 ml/ kg
8	Seedkare Orange (seed dye)	1 ml/ kg
9	Untreated control	-

Each treated and untreated sub-lot was again divided into 3 sub-sub-lots of 20 grams each. These three sub-sub-lots were stored in 700-

gauge polythene bag. This was done so that in one observation only one sub-sub-lot is taken without disturbing the rest of the stored seed. All the packets were stored at the ambient condition at the Division of seed Science & Technology, IARI, New Delhi from October 2003 to April 2004.

Efficacy of chemical seed treatment against *Bipolaris oryzae* and other seedborne fungi evaluated based on seed germination test, standard blotter method (ISTA 2003) in vivo and inhibition zone technique in vitro (Nene and Thanpliyal 1993).

**Seed Germination test:** The germinability of seed was determined by using between paper methods (ISTA 2003). Four hundred seeds in four replicates of hundred seeds each were placed between two layers of moist germination papers with the help of counting board. Then the germination papers were folded along one edge and then rolled up carefully ensuring that no excess pressure is placed on the seeds. These were wrapped with the sheet of wax paper to reduce surface evaporation and placed in a germinator at 25<sup>o</sup> C temperatures in an upright position. After 7 days' incubation, the seedlings were evaluated for normal, abnormal seedlings, fresh ungerminated and dead seeds according to the International Rules for Seed Testing (2003). A second count of abnormal seedlings was made on completion of the test after 14 days. However, germination percentage was recorded based on normal seedlings only.

**Standard blotter method:** Incidence of different fungi associated with the seeds was followed by the standard blotter method (ISTA 2003). For each seed sample, four hundred seeds were used. Twenty-five seeds per plate were placed in a plastic Petri plate of 10 cm diameter containing three well-moistened blotters. These plates were then incubated at 20°C ± 1°C under alternating cycle of 12 h of darkness and 12 h light under Near Ultra Violet (NUV) for seven days. After 7 days' incubation, the seeds were examined under stereo binocular microscope for the presence of associated seedborne fungi, if needed were confirmed with the aid of compound microscope and the pertinent literature. Total number of seeds infected by

specific seedborne fungus was scored to determine percentage of seed infection.

**Inhibition zone technique:** In this technique, known amount ( $10^5$  spores/ml) of spore suspension is mixed with 1000 ml of PDA. Dose of chemicals used for seed treatment was considered as the assumption of 40,000 rice seeds per kg. Amount of residues present on each seed is expected to be in the range of 0 µg to 62.5 µg in case of Mancozeb, Thiram, Carbendazim and Vitavax, and 0 µl to 0.025 µl in case of Neem oil, Palmarosa oil, Polykote and Seedkare Orange. Hence, an experiment was conducted by taking five concentrations (12.5, 25.0, 37.5, 50.0 and 62.5 µg) of Mancozeb, Thiram, Carbendazim and Vitavax, and taking five concentrations (0.005, 0.010, 0.015, 0.020 and 0.025 µl) in case of Neem oil, Palmarosa oil, Polykote, Seedkare Orange. These chemicals with a range of concentrations were placed in the center of the Petri plate having test organism and further incubated at  $20 \pm 1^\circ\text{C}$  temperature for 4-5 days under normal conditions of 12 h light (NUV) and 12 h darkness. The inhibition zones developed around the seeds were measured to the nearest mm by template, and then by plotting a standard curve. During the actual experiment, treated seeds were placed in the center of fungal colony in four replicates. The inhibition zones developed around the seeds were measured with the help of standard curve to assess the quantity of fungicides and chemicals remained on the seeds.

## RESULTS AND DISCUSSION

### Efficacy of chemical seed treatment in vivo

#### *Effect of seed treatment on germination*

Effect of seed treatment on germination after storage up to 6 months was illustrated in Fig. 1.

The fall in germination during storage was gradual and slow in seeds treated with Vitavax, Mancozeb & Thiram after 2, 4 and 6 months of storage, but all of these treated seeds retained above the Minimum Seed Certification Standard (MSCS) of 80% germination. However, germination in treatments with Neem oil, Palmarosa oil, Polykote, Seedkare Orange and the untreated

control declined below the MSCS of germination after 6 months of storage, especially the seeds treated with Palmarosa showed the lowest germination values of 69.88 percent after 6 months of storage, it may be due to its phytotoxic nature.

#### *Effect of seed treatment on seedborne fungi*

Ten seedborne fungi were found to be associated with seed lots before seed treatment, in which *Bipolaris oryzae*, *Alternaria padwickii* and *Curvularia lunata* were predominant whereas *Fusarium moniliforme*, *Fusarium pallidoroseum* [*Fusarium semitectum*], *Alternaria alternata*, *Sarocladium oryzae*, *Aspergillus* spp., *Penicillium* spp. and *Rhizopus* spp. found inconsistently in the seed lots. Efficacy of chemical seed treatment is illustrated in details for each seedborne fungus as follows:

***Bipolaris oryzae*:** The decrease in incidence of *Bipolaris oryzae* during storage was strong in seeds treated with Vitavax, Mancozeb and Thiram, as it was 0.69, 1.5 & 0.75 percent after 2 months of storage which came down to 0.13, 0.5 & 0.25 percent after 6 months of storage respectively. Bavistin and other chemicals did not affect the fungus (Fig. 2)

***Alternaria padwickii*:** The decline in incidence of the fungus during storage was slope in seed treated with Vitavax, Mancozeb, Thiram as it was 1.19, 1.13, 0.63 percent after 2 months of storage which came down to 0.63, 0.5, 0.13 percent after 6 months of storage, respectively. While the mean incidence of the fungus in untreated control was 13.44, 9.44 and 6.88 percent after 2, 4 & 6 months of storage, respectively. The effect of other treatments on incidence of the fungus was slightly and inconsistently significant (Fig. 3)

***Curvularia lunata*:** The fall in incidence of *Curvularia lunata* during storage was steep in seed treated with Vitavax, Mancozeb and Thiram as it was 0.63, 1.0, and 0.81 percent after 2 months of storage. Vitavax, Mancozeb and Thiram came down gradually to 0.44, 0.63 and 0.5 percent after 6 months of storage, respectively. But the mean incidence of the fungus in untreated control was 11.63, 9.25, and 7.06 percent after 2, 4 and 6 months of

storage, respectively. The effect of other treatment on incidence of the fungus was slightly significant; they ranged from 3.88 – 9.13 percent (Fig. 4)

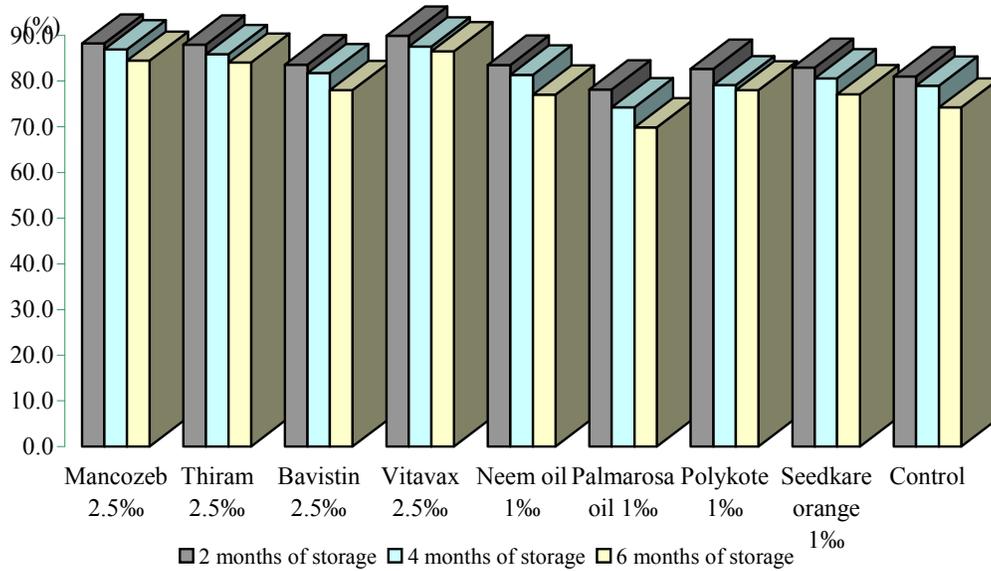


Fig. 1: Effect of seed treatment on germination

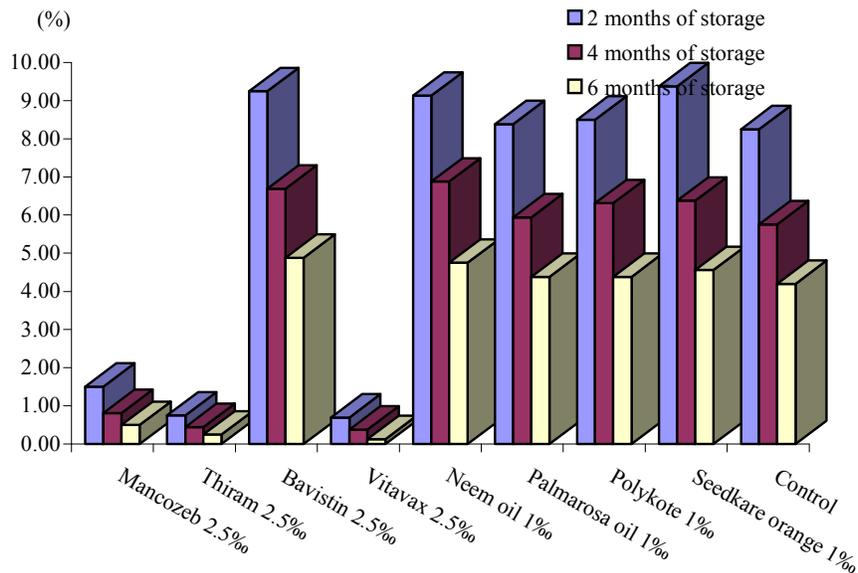


Fig. 2: Effect of seed treatment on incidence of *Bioplaris oryzae*

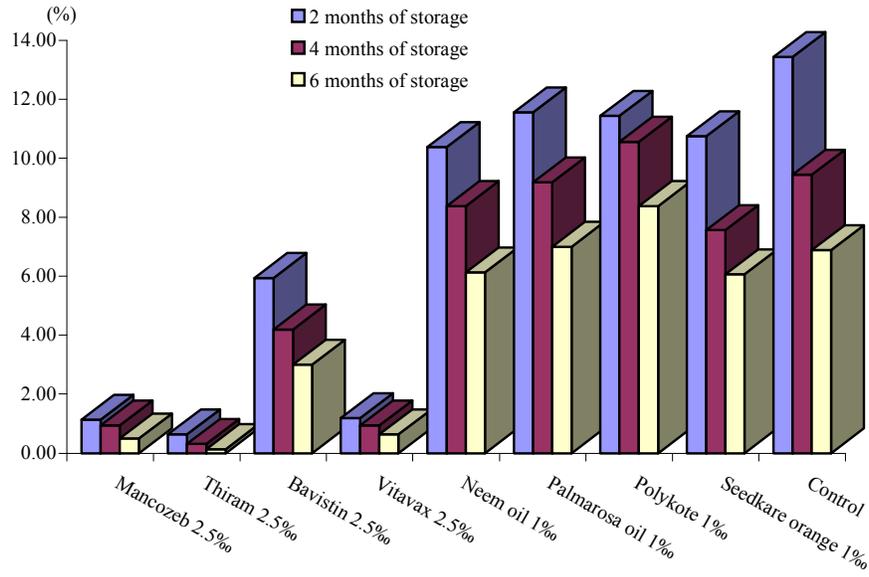


Fig. 3: Effect of seed treatment on incidence of *Alternaria padwickii*

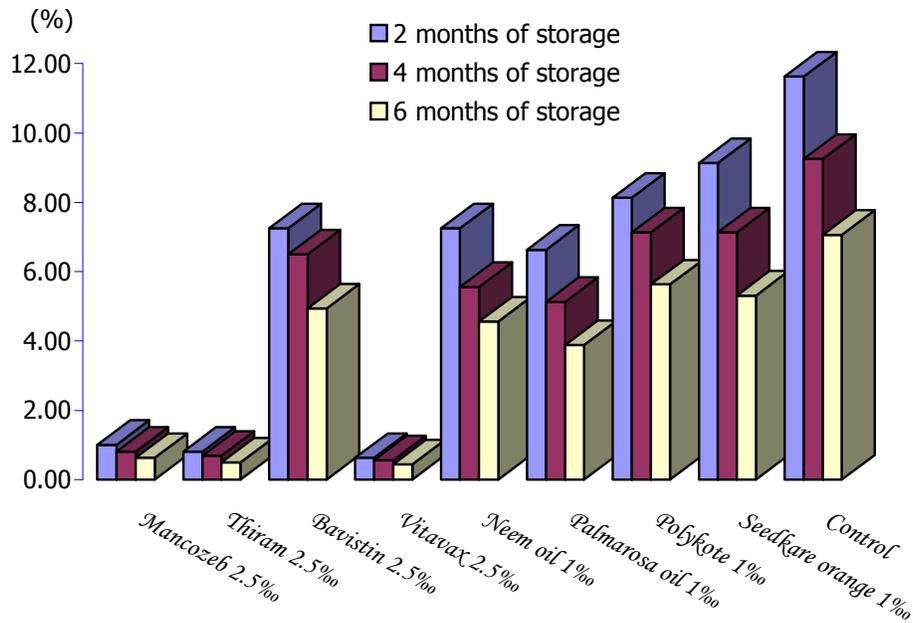


Fig. 4: Effect of seed treatment on incidence of *Curvularia lunata*

**Other seed borne fungi**

Vitavax, Thiram, Mancozeb all proved to be effective against *Fusarium moniliforme*, *Fusarium pallidoroseum*, *Sarocladium oryzae*, *Alternaria alternata*, *Aspergillus* spp., *Penicilium* spp. and *Rhizopus* spp. ranged 0.19 – 0.25%, 0.75 – 1.88%, 0.63 – 1.57%, 1.56 – 1.81%, 0.73 – 0.98% and 1.25 – 5.06%, respectively. Other chemicals viz. Bavistin, Neem oil, Palmarosa oil, Polykote and Seedkare orange were not effective against these fungi.

In general, mean incidence of field seedborne fungi viz. *Bipolaris oryzae*, *Alternaria padwickii*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium pallidoroseum* and *Sarocladium oryzae* declined progressively during 6 months of storage. However, incidence of storage fungi viz. *Aspergillus* spp., *Penicilium* spp. and *Rhizopus* spp. increased during seed storage for 6 months. The incidence of *Penicillium* spp. and *Aspergillus* spp increased during the 12 months of their experiment (Macedo et al. 2002). Similarly, Pratima and Ray (1998) revealed a gradual decrease in field fungi and an immediate increase in storage fungi in 10 rice varieties, when seeds were stored for 180 days. Vitavax, Thiram and Mancozeb were fungicides that performed best in reducing incidence of almost seedborne fungi. The results were confirmed by authors viz. Kauraw (1986), Rao and Ranganathajah (1988), Pasha et al. (1991), Chhetry (1993), Sachan and Agarwal (1994), Sistema and Ronco (1994), Rahman et al. (2000), Countinho et al. (2000), and Parisi et al. (2001). However, Bavistin was not effective against *Bipolaris oryzae* and this result compromised with the finding of Kannaiyan and Radhakrishnan (1982), Rao and Ranganathajah (1988). Neem oil was not effective against *Bipolaris oryzae* and other fungi, but Rajappan et al. (2000) found that

neem oil inhibited *Pyricularia oryzae*, *Sarocladium oryzae* and *Helminthosporium oryzae*. Palmarosa oil was not effective against seedborne fungi and this has not presently proved in literature. Seed dyes viz. Polykote and Seedkare Orange were mostly not effective against seedborne fungi as compared the untreated control. There is no information available in the literature about the effect of these seed dyes on seedborne fungi.

**Efficacy of chemical seed treatment in vitro****Assessment of chemicals persisting on seed after storage**

Persistence of residues on the seed surface after two, four, and six months of storage was made to choose the best fungicide for seed treatment. *Bipolaris oryzae* was chosen as test organism due to its potential pathogenicity and seed lot as IR40750-R because of its high germination and low infection of seedborne fungi. Inhibition zone tests for plotting the standard curve and evaluating the chemical residues on the seed surface were described in method.

**Inhibition zone test for standard curve**

Out of eight chemicals used in the study, there were only three fungicides exhibited clear inhibition zones, even when they were used with a very small dose at 12.5 µg/seed. However, Vitavax was the best fungicide for seed treatment, followed by Thiram and Mancozeb as the mean areas of inhibition zones were 1105 mm<sup>2</sup>, 922 mm<sup>2</sup> and 435 mm<sup>2</sup> produced by them respectively. Other chemicals showed no inhibition zone area. High doses of these effective fungicides produced bigger areas of inhibition zone. These data and standard curves were demonstrated in Fig. 5

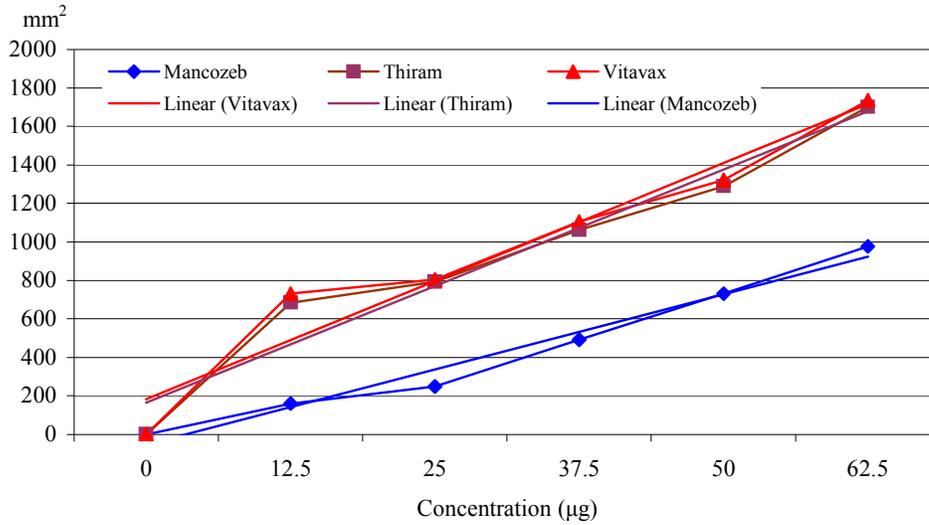


Fig. 5: Standard curves of inhibition zones

***Inhibition zone test for chemical residues on the seed surface***

Treated seeds of rice line IR40750-R were tested on inhibition zone technique at 0, 2, 4 and 6 months of storage to evaluate the chemical residues on the seed surface. Results of the study were the same as ones for standard curve. Vitavax, Thiram and Mancozeb exhibited clear inhibition zones, which decreased gradually during seed

storage, whereas other chemicals did not express any inhibition zone. Vitavax gave largest inhibition zone at 0, 2, 4 and 6 months of storage by 1698, 1644, 1591 and 1504 mm<sup>2</sup> respectively. Similarly, Thiram gave an inhibition zone of 1627, 1573, 1453 and 1386 mm<sup>2</sup> after 0, 2, 4 & 6 months of storage while Mancozeb gave 908, 870, 707 and 606 mm<sup>2</sup> inhibition zone after 0, 2, 4 & 6 months of storage, respectively (Fig. 6 and plate 1).



- First row (left to right)
- 1- Mancozeb 2.5g/kg
- 2- Thiram 2.5g/kg
- 3- Bavistin 2.5g/kg
- Second row (left to right)
- 4- Vitavax 2.5g/kg
- 5- Neem oil 1ml/kg
- 6- Palmarosa oil 1ml/kg
- Third row (left to right)
- 7- Polykote 1ml/kg
- 8- Seedkare orange 1ml/kg
- 9- Untreated control

Plate 1: Inhibition zones developed around the treated seeds at 6 months of storage

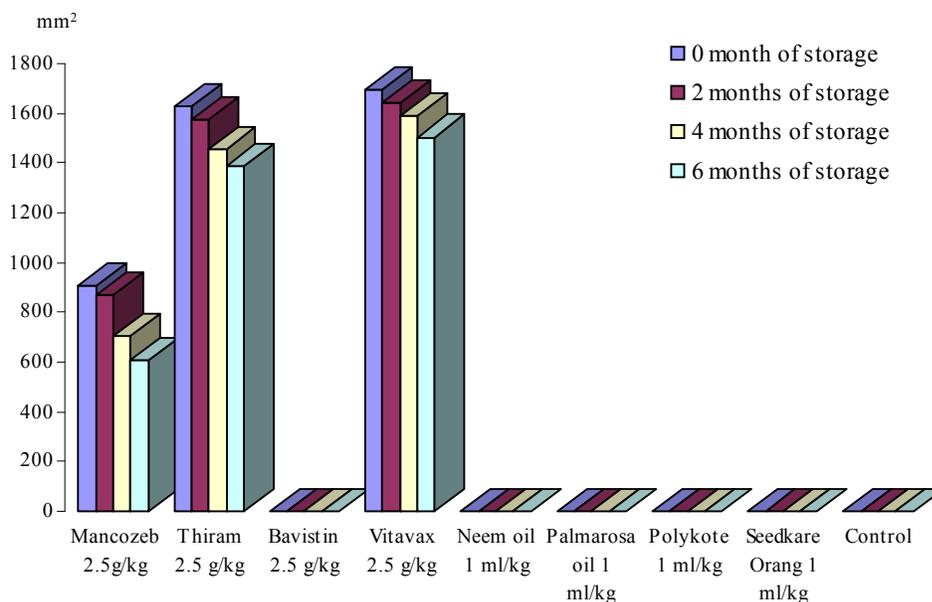


Fig 6: Inhibition zone on treated seed (mm<sup>2</sup>)

This showed that the quantity of Vitavax, Thiram and Mancozeb retained on seed surface ranged from 54.71 to 61.79 µg/seed, 51.56 to 60.53 and 41.18 to 61.71 µg/seed, respectively (Fig. 7).

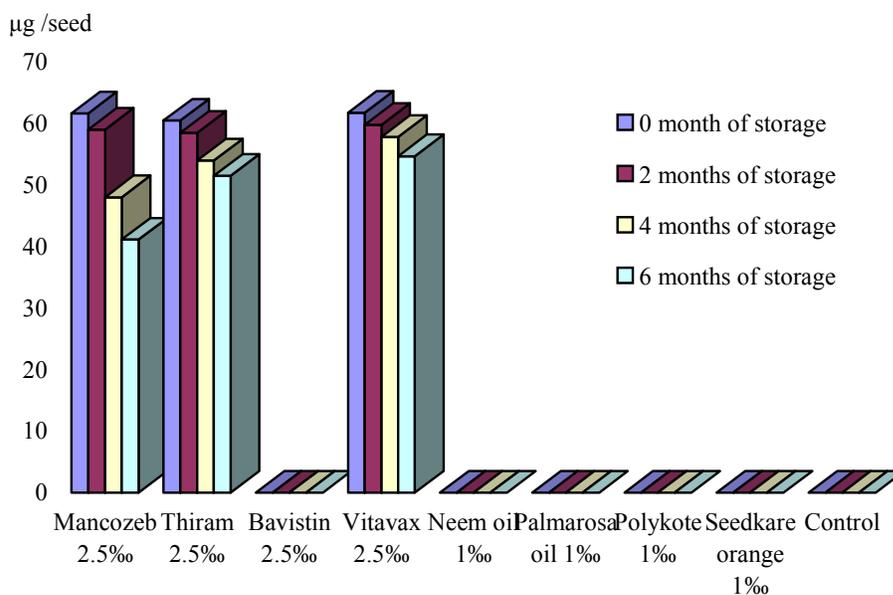


Fig 7: Estimated residue of chemical on individual seed (µg)

In general, three out of eight chemicals used for seed treatment viz. Vitavax, Thiram and Mancozeb were biologically active and have retained fungicidal properties against the testing organism *Bipolaris oryzae* even after 6 months of storage. The result was confirmed

by Dharam Vir and Sharma (1986) when they found that fungicides were active even after 20 years of storage. Other chemicals viz. Bavistin, Neem oil, Palmarosa oil, Polykote and Seedkare Orange did not perform any fungicidal properties against *Bipolaris oryzae*.

However, Rajappan et al., (2000) reported that Neem oil inhibited *Pyricularia oryzae*, *Sarocladium oryzae* and *Helminthosporium oryzae*.

### CONCLUSION

Seeds treated with Vitavax, Thiram and Mancozeb maintained germination above MSCS ( $\geq 80\%$ ) after six months of storage, while other chemicals could not retain germination above MSCS. Palmarosa oil caused deterioration in germination below MSCS, it was due to its phytotoxic nature. Seed dyes viz. Polykote and Seedkare Orange did not harm to germination.

Vitavax, Thiram and Mancozeb eradicated most of the seedborne fungi viz. *Bipolaris oryzae*, *Alternaria padwickii*, *Curvularia lunata* and other seedborne fungi. Whereas Bavistin was only slightly effective to *Alternaria padwickii* and *Curvularia lunata*. However, similar to other chemicals did not affect to *Bipolaris oryzae* and other seedborne fungi. Field fungi decreased progressively, while storage fungi increased gradually during the increasing period of storage.

In the inhibition zone test, only Vitavax, Thiram and Mancozeb produced inhibition zone areas, they maintained considerable chemical residues on individual seed after six months of storage by 87.5, 82.5% and 65.9% as compared to the initial quantity of 62.5  $\mu\text{g}$  at seed treatment.

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### Ảnh hưởng của xử hạt đến chất lượng hạt giống lúa *Oryza sativa* L.

Tám lô hạt giống được thu thập từ Bộ môn Khoa học & Công nghệ hạt giống, Viện nghiên cứu Nông nghiệp Ấn Độ, New Delhi năm 2003. Các lô hạt giống này được xử lý với tất cả tám loại hoá chất như sau: Xử lý 2.5g thuốc trừ nấm Mancozeb 50 WP, Thiram 75 SP, Bavistin 50 WP và Vitavax Powder cho 1 kg lúa, 1 ml dầu Neem, dầu Palmarosa, Polykote và Seedkare Orange cho 1 kg lúa, ngoài ra có một nghiệm thức đối chứng không xử lý thuốc. Các lô hạt giống sau khi được xử lý thuốc sẽ được tồn trữ trong bao PE có độ kín 700 gauge dưới điều kiện nhiệt độ trong phòng cho đến 6 tháng. Sau thời gian tồn trữ 2, 4, và 6 tháng các lô hạt giống được xử lý thuốc này sẽ được đánh giá về khả năng nảy mầm, chỉ số các loại nấm bệnh trên hạt và nấm kho vữa, ngoài ra còn đánh giá khả năng ức chế sự phát triển của nấm bệnh đốm nâu *Bipolaris oryzae* trong môi trường đĩa Petri PDA của các loại thuốc trừ nấm và các hóa chất bằng phương pháp thử nghiệm inhibition zone test (Nene, Y.L. and Thapaliyal, P.N., 1993). Kết quả các thí nghiệm như sau:

Các lô hạt giống được xử lý với các loại thuốc trừ nấm như: Vitavax, Thiram và Mancozeb đều có khả năng duy trì tỷ lệ nảy mầm trên mức chuẩn 80%, các loại hóa chất và thuốc trừ nấm còn lại đều không có khả năng duy trì tỷ lệ nảy mầm trên mức chuẩn 80%, đặc biệt dầu Palmarosa còn gây giảm tỷ lệ nảy mầm rất nặng, có thể loại dầu này có tính độc đối với mầm lúa.

Hầu hết các loài nấm trên hạt như *Bipolaris oryzae*, *Alternaria padwickii*, *Curvularia lunata* và nấm kho vữa như *Aspergillus* spp., *Penicillium* spp đều bị các loại thuốc trừ nấm như Vitavax, Thiram và Mancozeb ức chế hoặc tiêu diệt mạnh, trong khi đó Bavistin cũng là một loại thuốc trừ nấm phổ rộng nhưng không có khả năng hạn chế các loài nấm trên. Các loại hoá chất còn lại đều không có hiệu lực phòng trừ các loại nấm trên. Các loài nấm trên hạt được lây nhiễm từ ngoài đồng bị giảm tỷ lệ bệnh dần, trong khi đó các loài nấm kho vữa lại gia tăng trong suốt quá trình tồn trữ hạt

Các loại thuốc Vitavax, Thiram và Mancozeb đều tạo ra vùng ức chế sinh trưởng nấm rất lớn và rõ trên môi trường PDA đã chủng nấm *Bipolaris oryzae* và vẫn duy trì hiệu lực thuốc sau thời gian tồn trữ 6 tháng là 87,5% , 82,5% và 65,9% tương ứng với thứ tự các loại thuốc trên. Các loại thuốc còn lại đều không tạo ra được vùng ức chế sinh trưởng nấm, nên không có hiệu lực phòng trừ loại nấm này.