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Antagonism between fungicide-insecticide treatments and dietholate in irrigated rice seeds[1]

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ABSTRACT – The plant safener dietholate can be used for seed treatments (ST) to improve the selectivity of clomazone in rice cultures, thus allowing the application of higher dosages of this herbicide. The objective of this research was to evaluate the interaction between seed fungicide-insecticide treatments with and the plant safener dietholate in irrigated rice. Three experiments were carried out, and the first of them was conducted in a laboratory, following a completely randomized experimental design with four replications. Different ST and treatment-to-sowing intervals were evaluated. The second and third experiments were performed in field conditions, in a completely randomized block design with four replications. In experiment two, seeds were treated with different fungicides, in association or not with dietholate; while in experiment three, the effect of the ST with fungicides and dietholate with clomazone application were assessed. The results evidenced that the association between carboxin + thiram + dietholate reduced seed germination, vigor, and the dry mass of shoot of rice plants. The longer it takes between ST and sowing, the more rice seed germination is negatively affected. The association between fungicides and dietholate may have been antagonistic, and thus interfered with the culture growth potential.

Index terms: fungicide, carboxin+thiram, *Oryza sativa* L., clomazone, safeners.

Antagonismo entre tratamentos com inseticidas, fungicidas e dietholate em sementes de arroz irrigado

RESUMO – Para proporcionar maior seletividade do herbicida clomazone à cultura do arroz, pode-se utilizar o protetor dietholate em conjunto com outros tratamentos de sementes (ST), o que possibilita o uso de doses maiores do herbicida. O objetivo do trabalho foi avaliar a interação entre o tratamento de sementes que utilizam fungicidas e inseticidas e o protetor dietholate em arroz irrigado. Foram realizados três experimentos, sendo que o primeiro foi conduzido em laboratório, em delineamento experimental inteiramente casualizado com quatro repetições. Neste caso, testou-se diferentes ST e intervalo em dias entre o tratamento de sementes e a semeadura. O segundo e o terceiro experimentos foram realizados em campo, seguindo um delineamento experimental de blocos casualizados com quatro repetições. No experimento dois, foram testados diferentes fungicidas nos ST com ou sem a adição de dietholate; enquanto no experimento três avaliou-se o efeito dos ST com fungicidas e dietholate, quando aplicados juntamente com clomazone. Os resultados evidenciaram que a associação carboxin + thiram + dietholate reduziu a germinação, o vigor de sementes e a matéria seca da parte aérea de plantas de arroz irrigado. O aumento no tempo entre o ST e a semeadura reduziu a germinação de arroz. A associação de fungicidas em combinação com dietholate no ST pode ter ação antagônica e interferir no estabelecimento da cultura.

Termos para indexação: fungicida, carboxin+thiram, *Oryza sativa* L., clomazone, protetores.

Introduction

Rice (*Oryza sativa* L.) is considered one of the most important food, once it constitutes the base of diet for more than three billion people (SOSBAI, 2016). Nowadays, rice crops are planted over around 168 million hectares worldwide,

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thus being the second most cultivated cereal, with an annual production of 741 million tons (SOSBAI, 2016). Brazil places among the ten biggest rice-producing countries (EPAGRI, 2017), and it harvested approximately 13 million tons in 2016/2017 (CONAB, 2017). Within the country, the states of *Santa Catarina* (SC) and *Rio Grande do Sul* (RS) together respond for 76% of the national production. *Rio Grande do Sul* alone accounts for 67% of it, with a total cultivated area of 1.1 million hectares and an average yield of about 8 tons. ha^{-1} (IRGA, 2018).

A yield-limiting factor in irrigated rice is the competition with weeds for environmental resources (SOSBAI, 2016). The intensive use of the monoculture system in low lands has contributed for increasing the weed population and elevating the resistance to herbicides. Cases of increased resistance have already been reported for troublesome plants that occur in irrigated rice crops in Brazil, such as weedy rice (*Oryza sativa*), sedges (*Cyperus difformis* and *Cyperus iria*), grasslike fimbry (*Fimbristylis miliacea*), California arrowhead (*Sagittaria montevidensis*), and common barnyard grass (*Echinochloa crus-galli*) (Heap, 2017).

Among the several forms of controlling weeds, the chemical method is largely employed by farmers, either as a pre- or post-emergence treatment. Clomazone is a pre-herbicide used before rice emergence, which oxidizes and produces 5-keto clomazone (Ferhatoglu et al., 2005). This compound represents the herbicide active-principle and inhibits the enzyme deoxy-xylulose-5-phosphate synthase (DXS), responsible for the isopentenyl pyrophosphate (IPP) synthesis route of the methylerythritol 4-phosphate (MEP) pathway. IPP is an important precursor involved in the biosynthesis of carotenoids in plants (Ferhatoglu and Barret, 2006). Sensitive plants that have been exposed to this chemical suffer from leaf bleaching due to loss of the green color (Senseman, 2007).

Important factors for clomazone selectivity are connected either with the cultivars or the dosages applied (Sherder et al., 2004). An alternative to boost the selectivity of this herbicide is the use of plant safeners, which increase the tolerance of the culture of interest, without reducing the weed control activity (Oliveira Junior and Inoue, 2011). The seed safener dietholate is used in rice plantations because it inhibits the cytochrome P-450 monooxygenase enzyme complex, which is responsible for the oxidation of clomazone in the mesophyll cells (Ferhatoglu et al., 2005). In regular conditions, the cytochrome P-450 monooxygenase works as a detoxication agent in plants; however, when clomazone is present, this enzyme complex causes its activation (Ferhatoglu et al., 2005; Yun et al., 2005).

Usually, rice seed treatment aims at controlling phytopathogens and insects, so that the crop can establish in the field. It must also be able to control soil-associated pathogens, fungi that grow during storage, and phytopathogens that attack in early crop stages. Thus, seed treatment is fundamental to assure the plant stand formation and a high yield (Menten and Moraes, 2010). However, there is not enough information on the possible interactions between fungicide-insecticide treatments and the safener dietholate. Also, little is known about factors related to the exposure time of seeds to the chemical treatment, which may pose a risk during storage due to phytotoxicity buildup (Menten, 1996).

A phytosanitary treatment of seeds is crucial for preventing diseases and insect infestation and, in many situations, it is performed by the joint application of dietholate. On that account, this study aimed at evaluating the interaction between seed fungicide-insecticide treatments and the chemical safener dietholate in irrigated rice.

Material and Methods

The study consisted of laboratory and field experiments divided into three parts and conducted between 2015 and 2017.

Laboratory experiment (Experiment 1)

The first experimental step was carried out from September 2015 to March 2016. It included the analysis of effects of different treatments on germination and vigor of rice seeds. For the trials, the cultivar IRGA 424 RI was chosen, and the tests were performed according to a completely randomized design, with four replications of 100 rice seeds. The seeds were sown in paper rolls that had been moistened with distilled water, in a 2-time the dry paper weight ratio. Then, the rolls were kept inside germinator set at an average temperature of 25 ºC for 12 days, until the moment to perform the germination count (Brasil, 2009).

The procedures were arranged in a factorial scheme, in which the factor A comprised the seed treatments (ST), as described in Table 1, and factor B included the intervals between the treatments and the sowing date. The levels considered in factor B were 0, 29, 60, 120, and 210 days after treatments (DAT). The previously mixed products were applied to the seeds at a relative volume of slurry of 18 mL (kg of seed)$^{-1}$. Such a value is the maximum slurry amount recommended for treating seeds with dietholate.

The variables in consideration were germination and seed vigor, as stated by Rules for Seed Testing (Brasil, 2009). The germination percentage was calculated by the quantification of normal seedlings, which corresponded to seeds germinated 12 DAS (days after sowing). The vigor was quantified through the cold test without soil, as proposed by Cicero and Vieira

(1994). According to their methodology, before placing the paper rolls with the seeds inside a germinator at a controlled temperature, they were stowed in plastic bags and let inside a cold chamber at a controlled temperature of 10 ºC for seven days. The results were quantified as aforementioned, and they were expressed as percentage of normal seedlings.

The resulting data were subjected to the ANOVA test ($p \leq 0.05$) and, once a significant difference was detected, they were analyzed through linear regression. Additionally, the results of the seed treatment factor were compared to each other by using the orthogonal contrast method ($p \leq 0.05$).

Field experiments (Experiments 2 and 3)

The experiments 2 and 3 were conducted in the field, during the 2016/2017 harvest. They were performed according to a randomized block design, with four replications.

The cultivar IRGA 424 RI was used in a sowing density of 100 kg. ha^{-1}. The seeds were planted on September 21st, 2016, in plots dimensioned 1.53 m x 5 m, with a useful area of 4.8 m², according to a minimum-cultivation system.

Table 1. Commercial products, active ingredients, and dosages used in the seed treatments (ST).

Treat.	Commercial product	Active ingredients (ai.)	Dosage (g ai 100 $kg.seeds^{-1}$)
1[1]	Standak top®	Pyraclostrobin Thiophanate methyl Fipronil	3.75 33.75 37.50
2[1]	Standak top® + Permit®	Pyraclostrobin Thiophanate methyl Fipronil Dietholate	3.75 33.75 37.50 480.00
3[1,2]	Maxim XL®	Fludioxonil Metalaxyl−m	2.00 5.00
4[1,2,3]	Maxim XL® + Permit®	Fludioxonil Metalaxyl−m Dietholate	2.00 5.00 480.00
5[1]	Maxim XL® + Cruiser Opti®	Fludioxonil Metalaxyl−m Lambda-cyhalothrin Thiamethoxam	2.00 5.00 18.75 105.00
6[1]	Maxim XL® + Cruiser Opti® + Permit®	Fludioxonil Metalaxyl−m Lambda-cyhalothrin Thiamethoxam Dietholate	2.00 5.00 18.75 105.00 480.00
7[1,2]	Vitavax-thiram®	Carboxin Thiram	60.00 60.00
8[1,2,3]	Vitavax-thiram®+ Permit®	Carboxin Thiram Dietholate	60.00 60.00 480.00
9[1]	Vitavax-thiram® + Cruiser Opti®	Carboxin Thiram Lambda-cyhalothrin Thiamethoxam	60.00 60.00 18.75 105.00
10[1]	Vitavax-thiram® + Cruiser Opti® + Permit®	Carboxin Thiram Lambda-cyhalothrin Thiamethoxam Dietholate	60.00 60.00 18.75 105.00 480.00
11[1,2]	Without treatment	-	-

[1]Treatments used in experiment 1.
[2]Treatments used in experiment 2.
[3]Treatments used in experiment 3.

Fertilization was carried out at the moment of sowing, in a dosage of 400 kg. ha^{-1} of NPK fertilizer (formulated 04-17-27), as indicated by the soil analysis.

As for the experiment 2, the factor A tested different fungicides, chosen based on the results of the laboratory experiment, and untreated control (Table 1); whereas in factor B, the presence and absence of the plant safener dietholate (480 g ai. 100 kg. $seeds^{-1}$) were assessed. The treatments of this experiment did not receive the herbicide clomazone during the pre-emergence phase.

Experiment 3 employed a unifactorial scheme, including the following treatments: without fungicide and with dietholate, without fungicide but with dietholate (untreated control), and treatments four and eight selected from the first experiment (Table 1). Except for the control, the other treatments received an application of clomazone (720 g ai. ha^{-1}) during the pre-emergence phase.

The variables considered were these: shoot dry mass (DM), emergence speed index (ESI), population (POP), and plant height (HEI). To evaluate the DM, rice plants within a 0.25 m^2 area were collected 7, 14, and 21 days after emergence (DAE). The plants were cut at the soil level and then maintained inside an oven at 65 ºC until they reached a constant mass.

To establish the ESI through the equation of Popinigis (1977), the emerged seedlings within 1 m were counted daily until the stabilization of emergence. Seedlings were considered emerged when the shoot reached 1 cm long.

The features POP and HEI were assessed on the V4 stage. POP was determined by counting the seedlings within a 0.25 m^2 space, and HEI by gauging ten plants per plot with a millimeter ruler. In the latter case, the measurement considered the distance from the soil surface to the apex of the highest leaf with an extended blade. The final value corresponded to the mean of the sampled plants.

The gathered data were submitted to the ANOVA test ($p \leq 0.05$), and comparison-of-mean tests were performed whenever significant differences were detected among the variables. In the second experiment, the Duncan's test was applied to factor A ($p \leq 0.05$), and the T test to factor B ($p \leq 0.05$). In the third experiment, the Duncan's test was used ($p \leq 0.05$).

Results and Discussion

The analysis of variance evidenced interaction among the studied factors for both variables considered in experiment 1 (laboratory). As for experiment 2, the treatments showed interaction only for the variable DM, assessed 7 DAE. Still regarding this variable, the effect of dietholate prevailed 14 DAE. Last, in experiment 3, the treatment factors showed significance for the variables ESI and DM, evaluated 7 DAE.

Use of insecticides and fungicides in association with dietholate and the impact on germination and seed vigor (Experiment 1)

In general, the germination percentage reduced as the time interval between the seed treatment and the moment of planting increased (Figure 1). On average, such a delay lowered germination by 10% (Figure 1), which suggests that treatments should be performed right before sowing to prevent seed viability loss. Similar findings were obtained in soybean treated with insecticide and fungicide at different storage times. In this case, the active-principles might have had a phytotoxic effect on the seeds, thus compromising their germination capacity (Ludwig et al., 2011).

The application of dietholate overall affected seed germination at different levels (Figure 1). However, the most significant impact was observed in the carboxin + thiram + dietholate treatment, where the germination assessed 210 DAT was off by 20%, in comparison with the control without the safener (Figure 1). On that account, it is possible to infer that the combination of dietholate and carboxin + thiram shows antagonism; thus its use should be avoided, on the risk of harming the establishment of the rice culture.

Based on the orthogonal contrast analysis, it was possible to notice that the most significant negative impact on germination was caused by the association of dietholate with fungicides (Table 2). Within this group of chemicals, the germination reduced 16% due to the association with carboxin + thiram, similarly to the previous observations. This value was higher than that of the other seed treatments, which marked decreases of 5% and 9% in the associations with pyraclostrobin + thiophanate methyl + fipronil and fludioxonil + metalaxyl-m, respectively (Table 2).

In a similar study with irrigated rice, seed treatments with dietholate, both in isolated form or combined with other products, negatively influenced the germination and vigor of seeds in cold temperature conditions (Rosa et al., 2017). Thus, when the germination of the untreated seeds and the dietholate-free ones was contrasted, no significant difference was observed. Nevertheless, approximately 9% germination reduction was detected among seeds treated of dietholate, in comparison with the untreated ones (Table 2). Therefore, regardless of the ST used, the application of dietholate reduced the germination of rice seeds.

When the rice seeds were subjected to the vigor test, a reduction trend was noticed whenever the treatments with fungicide and insecticide were added to seed, regardless of the active ingredient in test, while a sharper reduction happened when was added the safener dietholate to them (Figure 2).

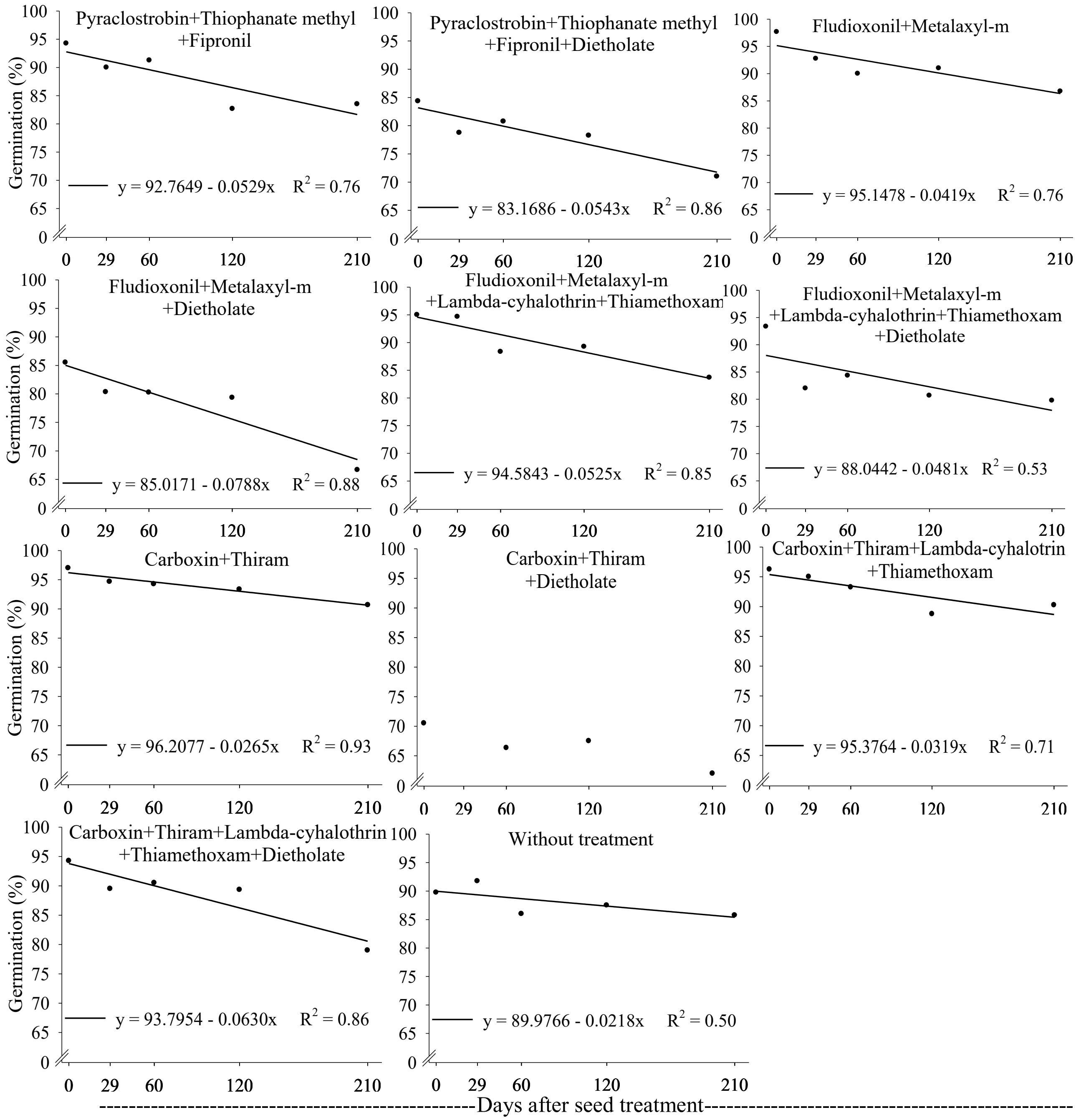

Figure 1. Germination (%) of rice seeds (*Oryza sativa* L.) subjected to different treatments and sowing dates after the treatments. The dots represent the average value of each treatment.

Likewise to what happened in germination, in contrast with the untreated seeds, the association carboxin + thiram + dietholate compromised the most the vigor of seeds, with roughly 20% loss detected already at the beginning of the trials (0 DAT) (Figure 2). Also, compared to the seeds treated only with carboxin + thiram, the addition of dietholate caused the vigor to diminish around 30% within 210 DAT (Table 3). This fact confirms that the association was harmful to rice seed viability, thus being considered a case of antagonism.

In a comparison between the vigor of untreated seeds and those that had received dietholate, it became evident an average reduction of 7% (Table 3), which suggests that applying the plant safener might have negatively influenced the establishment of rice. For a better growth performance, it is fundamental for rice plants to establishing fast and uniformly, so that they become more capable of competing for resources than the weeds (Concenço et al., 2007).

Initial establishment of irrigated rice seeds treated with dietholate (Experiments 2 and 3)

According to the field studies, the treatments containing the safener dietholate mixed with carboxin + thiram showed a significant diminution in the variable DM assessed 7 DAE, in comparison with the use of the fungicide alone (Table 4). The effect of dietholate in rice growth could still be observed 14 DAE, when the treatment with the safener, even without the application of the herbicide clomazone, decreased the DM of the shoots (Table 4). In general,

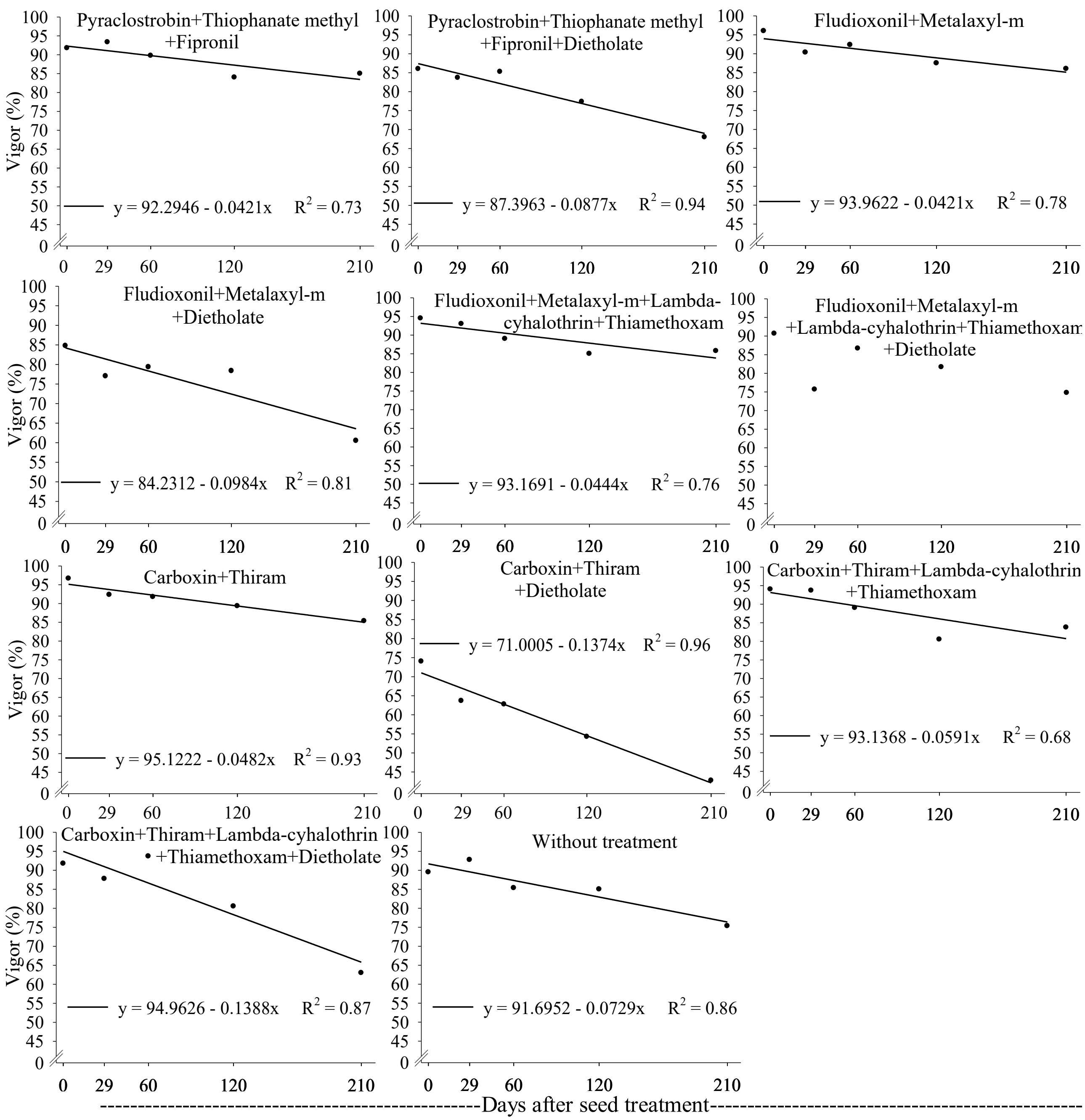

Figure 2. Vigor (%) of rice seeds (*Oryza sativa* L.) subjected to different treatments and sowing dates after the treatments. Dots represent the average value of each treatment.

Table 2. Orthogonal contrasts for germination (%) of irrigated rice seeds (*Oryza sativa* L.), as a function of the different treatments with fungicide, insecticide and dietholate, and sowing times.

Treatments	0 DAT[1]	29 DAT	60 DAT	120 DAT	210 DAT
Without dietholate X All	90^{ns} X 91	92* X 86	86^{ns} X 86	88* X 84	86* X 79
Pyraclostrobin+thiophanate methyl+fipronil without dietholate X Pyraclostrobin+thiophanate methyl+fipronil with dietholate	94^{ns} X 84	90* X 79	91* X 81	83^{ns} X 78	84* X 71
Fludioxonil+metalaxyl−m without dietholate X Fludioxonil+metalaxyl−m with dietholate	96* X 89	94* X 81	89* X 82	90* X 80	85* X 74
Carboxin+thiram without dietholate X Carboxin+thiram with dietholate	97* X 82	95* X 77	94* X 80	91* X 77	90* X 69
Fungicides without dietholate X Fungicides with dietholate	97* X 78	94* X 70	92* X 74	92* X 73	88* X 64
Insecticides+fungicides without dietholate X Insecticides+fungicides with dietholate	95* X 91	93* X 84	91* X 85	87* X 82	86* X 76
Fungicides X Insecticides+fungicides	86* X 93	83^{ns} X 88	84^{ns} X 88	82* X 85	76^{ns} X 81
Without dietholate X With dietholate	96* X 85	93* X 79	92* X 81	89* X 78	87* X 72
Without treatment X Without dietholate	90* X 96	92^{ns} X 93	86* X 92	88^{ns} X 89	86^{ns} X 87
Without treatment X With dietholate	90* X 85	92* X 79	86* X 81	88* X 78	86* X 72
CV (%)	2.49	3.56	1.93	2.83	4.42

[1] Days after seed treatment.
* and ^{ns} significant difference and non-significant difference, respectively ($p \leq 0.05$).

Table 3. Orthogonal contrasts for the vigor (%) of irrigated rice seeds (*Oryza sativa* L.), as a function of the different treatments with fungicide, insecticide and dietholate, and sowing times.

Treatments	0 DAT[1]	29 DAT	60 DAT	120 DAT	210 DAT
Without dietholate X All	90^{ns} X 90	93* X 85	85^{ns} X 86	85* X 80	75^{ns} X 74
Pyraclostrobin+thiophanate methyl+fipronil without dietholate X Pyraclostrobin+thiophanate methyl+fipronil with dietholate	92* X 86	93* X 84	90* X 85	84^{ns} X 77	85* X 68
Fludioxonil+metalaxyl−m without dietholate X Fludioxonil+metalaxyl−m with dietholate	95* X 87	92* X 76	90* X 83	86* X 80	86* X 68
Carboxin+thiram without dietholate X Carboxin+thiram with dietholate	95* X 83	93* X 77	90* X 76	84* X 67	84* X 51
Fungicides without dietholate X Fungicides with dietholate	93* X 90	93* X 83	89* X 88	83* X 80	85* X 69
Insecticides+fungicides without dietholate X Insecticides+fungicides with dietholate	87* X 92	81* X 88	81* X 89	76* X 82	68* X 78
Fungicides X Insecticides+fungicides	94* X 85	91* X 78	89* X 81	84* X 74	83* X 61
Without dietholate X With dietholate	90* X 94	93^{ns} X 91	85* X 89	85^{ns} X 84	75* X 83
Without treatment X Without dietholate	90* X 85	93* X 78	85* X 81	85* X 74	75* X 61
Without treatment X With dietholate	92* X 86	93* X 84	90* X 85	84^{ns} X 77	85* X 68
CV (%)	1.75	2.47	3.38	4.38	3.56

[1] Days after seed treatment.
* and ^{ns} significant difference and non-significant difference, respectively ($p \leq 0.05$).

Table 4. Effect of the seed treatments with fungicide and dietholate on the shoot dry mass (DM, g 0,25m^{-2}) of irrigated rice plants (*Oryza sativa* L.), assessed 7, 14, and 21 days after emergence (DAE).

Seed treatments (ST)	DM 7 DAE		DM 14 DAE		DM 21 DAE	
	Dietholate					
	With	Without	With	Without	With	Without
Control	0.34 Aa[1]	0.43 Aa	1.42[ns]	1.83	4.49[ns]	5.12
Metalaxyl	0.39 Aa	0.44 Aa	1.53	1.96	4.80	4.85
Carboxin + Thiram	0.28 Ab	0.67 Aa	0.71	1.91	4.73	3.86
Effects of the treatment factors						
Dietholate	0.34 b	0.51 A	1.22 b	1.90 a	4.67 [ns]	4.61
Control	0.39[ns]		1.62[ns]		4.81[ns]	
Metalaxyl	0.42		1.74		4.83	
Carboxin + Thiram	0.48		1.31		4.30	
CV(%)	26.05		32.59		32.44	

[1] Means followed by distinct uppercase letters, in the column, and lowercase letters, in the row, indicate a significant difference, according to Duncan's and T tests ($p \leq 0.05$).
[ns] no significant difference was observed, according to the F test ($p \leq 0.05$).

although dietholate initially affected the establishment of rice plants, such damage did not endure, once its effect was not remarked 21 DAE. A similar outcome was found in a study with three cultivars of beans. In this case, the application of higher dosages of dietholate, even without the joint use of clomazone, produced an adverse effect on the accumulation of DM in plant shoot (Takano et al., 2012).

The main effect of the dietholate use came from the interaction with other products, as evidenced by the treatment without fungicide + dietholate, in which the ESI observed was higher than that of treatments with associated fungicides (Table 5). This result corroborates the laboratory experiment findings, according to which no significant difference was noticed in vigor, nor the dietholate application posed harm to the seeds (Table 3). In this sense, despite the lower ESI of the treatment without fungicide + dietholate that did not receive clomazone, the DM assessed 21 DAE was superior to the other treatments, thus evidencing the phytotoxic effect of this herbicide at high dosages, even when the plant safener was also present (Table 5).

Results of the effect of dietholate in rice seed treatment showed a significant reduction in the ESI values when seeds also received the plant safener. In these cases, coating the seeds might have decelerated water absorption and, consequently, provoked alterations in metabolic e biochemical processes required for germination and emergence (Mistura et al., 2008). The outcomes of the present work suggest that the association between fungicides and the safener dietholate might influence in early phases of rice development. Therefore, it is worth remarking that treating rice seeds with dietholate can affect the establishment of the culture due to adverse effects from its interaction with certain fungicides.

Table 5. Effect of the seed treatments with fungicide and dietholate, in association with the pre-emergence application of clomazone, on the emergence speed index (ESI) and shoot dry mass (DM, g 0.25 m^{-2}) of irrigated rice plants (*Oryza sativa* L.), assessed 7, 14, and 21 days after emergence (DAE).

Treatment	ESI	DM 7	DM 14	DM 21
Without fungicide + dietholate	23.04 a[2]	0.45[ns]	1.39[ns]	4.01 b
Without fungicide + dietholate[1]	13.14 b	0.55	1.43	5.31 a
Metalaxyl + dietholate	17.06 b	0.72	1.65	3.85 b
Carboxin + Thiram + dietholate	15.23 b	0.61	1.32	3.30 b
CV (%)	19.19	29.09	22.94	14.49

[1] Treatment did not receive the herbicide clomazone dosage of 720g ai ha^{-1}
[2] Means followed by distinct letters in the column indicate a significant difference, according to the Duncan's test ($p \leq 0.05$).
[ns] no significant difference was observed, according to the F test ($p \leq 0.05$).

Conclusions

Using the safener dietholate in association with the seed treatments carboxin + thiram, pyraclostrobin + thiophanate methyl + fipronil, or fludioxonil + metalaxyl‒m exhibits antagonism with germination and vigor of irrigated rice seeds.

Adding dietholate to fungicide-insecticide treatments reduces the germination and vigor of rice seeds.

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