The Pharma Innovation Journal 2023; 12(9): 1069-1071

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(9): 1069-1071

www.thepharmajournal.com
Received: 17-06-2023
Accepted: 20-07-2023

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In vitro evaluation of insecticidal seed treatments on maize, *Zea mays* L.

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Abstract
Under its most ambitious effort, Total Seed Treatment Campaign 2007, the Indian government offers recommendations for seed treatment for a variety of crops. Three insecticides, imidacloprid (600 FS), fipronil (5 FS), and chlorpyriphos (20 EC) (each at three dosages), were evaluated *in vitro* (lab tests) to see whether test doses for the maize crop had any harmful effects. Statistics showed that seedling lengths were decreased after treatment with chlorpyriphos (P 0.05). All three imidacloprid test dosages (1.5 ml, 3 ml, and 5 ml per kg seed) increased overall length but statistically were comparable to the control. A substantial difference from all other treatments was seen in the case of the fipronil test-dose (7 ml/kg seed), which led to an increase in overall seedling length. Chlorpyriphos dosages all resulted in a sharp reduction in seedling length, indicating a negative effect on seedlings. Imidacloprid and fipronil seed treatments were shown to be safer and recommended for maize crops in the study's final analysis. Chlorpyriphos is not suggested for treating maize seeds, though. These seed-treatment rates might be tested for additional field validation in order to reduce pest insects that are of concern.

Keywords: Seed treatment, fipronil, imidacloprid, maize, and *Zea mays* L.

Introduction

The protection of developing seedlings from insects, diseases, and pests begins with seed treatment. Under its most ambitious effort, Total Seed Treatment Campaign 2007 (www.ppqs.gov.in), the Indian government offers recommendations for seed treatment for a variety of crops. In India, the application of seed treatments to field crops has significantly grown in recent years. Treatments applied to seeds offer protection against termites and other sucking insects including the shoot fly, aphids, and jassids in some areas. The majority of the time, recommendations for seed treatment are made based on field experiments; but, in rare instances, solely *in vitro* (=laboratory) studies are used as the basis for recommendations. This issue was addressed in our inquiry; maize crop *in vitro* investigations were conducted.

Materials and Methods

Seeds of maize (var. HQPM-1) were purchased from the IARI's Division of Genetics in New Delhi. As per the Tabale 1, the seeds were given three separate dosages of each of the following: chlorpyriphos 20 EC (Dursban®), fipronil 5SC (Regent®), and imidacloprid 48% FS (Gaucho® 600 FS). The test also included water as an untreated control. Insecticides were initially mixed with water to create the final amount needed to soak one kilogram of seed, which varied from 50 to 100 ml depending on the kind of crop. The necessary amount of the insecticide emulsion was then sprinkled over the seeds, which were then stirred often to achieve equal seed coating. The seeds were initially spread out on plastic trays. After 24 hours, a germination test was performed on the treated seeds.

By putting the seeds in folded envelopes that were positioned vertically in the germination chamber, the seeds were allowed to sprout between two layers of germination paper. Throughout the germination period, the chamber's temperature was maintained at 25 1 °C in accordance with ISTA recommendations. 100 seedlings from each treatment were sowed in the paper towel three times. According to ISTA (2008) [5], seedlings that were normal, aberrant, and ungerminated were counted 10 days after seeding. Ten seedlings were chosen at random, and their plumules, radicles, and overall lengths were measured.

Ten seedlings from each replication were observed to have mean values for (a) radicle and plumule length; and (b) dry weights (dry weight obtained after 24 hours in oven). These steps are used to determine vigor indices (VI):

Germination % x Mean root and shoot length (cm) is how you calculate the vigor index (I)

Germination % x Mean dry weight (g) = Vigor Index (II)

To support the causes of germination failure in diverse insecticidal seed treatments or to identify the impact of insecticides on seed viability, the Tetrazolium test was used to maize. After the usual germination test, ungerminated seeds of the two crops listed above were removed and put through the Rapid Tetrazolium Test (TZ).

Ungerminated maize seedlings were retrieved from the typical germination test and immersed in water for an hour. After that, seeds were split lengthwise. Half of each seed was added to a flask containing a salt solution of 2,3,5-triphenyl tetrazolium, which was maintained at 300C for 24 hours in the dark. After seeing the staining pattern, the seeds were categorized as viable or non-viable/dead seeds based on the location and size of the necrotic regions in the embryo. Hard seeds and dormant seeds are regarded as viable seeds as well (Agrawal, 1995) [1].

One factorial ANOVA was performed on the data using the industry-standard statistical software (OPSTAT online, www.hau.ernet.in/opstat.html).

Results and Discussion

Tables 1 show how different seed treatment chemicals (imidacloprid, fipronil, and chlorpyriphos, each at three dosages) affect the percentage of seeds that germinate, the length of seedlings, and the vigor indices on the seed germination of maize crops.

The recorded germination rate for untreated (control) seeds is 96.88 percent. Fipronil (two dosages, 5 ml and 7 ml/kg seed) and imidacloprid (all three test doses) had germination rates over 90%, which ISTA (2008) [5] recommends as the minimum seed germination criteria for maize hybrids. However, all three test dosages of chlorpyriphos exhibited germination rates of less than 90%, and other seedling metrics were also negatively impacted; as a result, this study came to the conclusion that chlorpyriphos is not advised for use as a seed treatment in maize.

Total seedling length (average value) for the control was 38.88 cm, but the range for various insecticidal seed treatments is 25.85–42.81 cm. Statistics showed that seedling lengths were decreased after treatment with chlorpyriphos (P 0.05). All three imidacloprid test dosages (1.5 ml, 3 ml, and 5 ml per kg seed) increased overall length but statistically were comparable to the control. A substantial difference from all other treatments was seen in the case of the fipronil test-dose (7 ml/kg seed), which led to an increase in overall seedling length. Chlorpyriphos dosages all resulted in a sharp reduction in seedling length, indicating a negative effect on seedlings.

According to the corresponding VI values, imidacloprid caused increases in both VI-I and VI-II that were substantially different from the control, indicating a potential phytotonic influence on growth parameters. Both vigor indices are comparable to control for the fipronil test dosage (5 ml/kg seed), however for the test dose (7 ml/kg seed), VI-I rose but VI-II fell dramatically. Both vigor indices significantly decreased when seedlings were treated with chlorpyriphos, along with other seedling characteristics.

The observed seedling metrics (such as seedling lengths and vigor indices) demonstrate a dose-dependent increase in the recorded VIs for three test dosages of imidacloprid, indicating a potential phytotonic action. As documented by Jarande and Dethe (1994) [11] in cotton, imidacloprid has a phytotonic impact that accounts for the higher seed quality metrics seen with seeds that have been treated with it. In both test crops, untreated seeds (control) offered just 1% dead seeds in the fast tetrazolium test (TZ). As shown in Table 2, seeds treated with chlorpyriphos showed a significant increase in dead seeds (13, 20%) at higher dosages (7 and 10ml/kg). Chlorpyriphos-treated seeds with dead seeds remained colorless. Therefore, dead seeds point to chlorpyriphos' phytotoxic effects when used at greater levels. Higher dosages (7, 10 ml/kg) of fipronil treatments also showed phytotoxicity, however with a smaller impact than chlorpyriphos. In contrast to the test-insecticides mentioned above, imidacloprid had no negative effects on the viability of seed. Despite a roughly two to three fold increase in dosage (from 1.5 to 5 ml/kg), no phytotoxicity was seen since the percentage of dead seeds increased so little (just by 1-2%).

Few scientists have studied the impact of chemical seed treatments on other cereal crops, such as *in vitro* wheat seed germination. In eight wheat cultivars, Kashyap *et al*. (1994) [6] looked at the impact of seed treatment, storage time for treated seeds, aberrant seedlings, ungerminated seeds, vigor index, and rate of germination. Between multiple test cultivars and test pesticides, they discovered a substantial variance. To insecticidal seed treatment (chlorpyriphos, aldrin, endosulfan, and formothion in various dosages), cultivar 'Sonalika' was most susceptible, while 'HD 2329' was least sensitive. Several researchers have shown that pesticides have a negative impact on seed germination (Scopes, 1969; Gifford *et al*., 1959) [8, 4]. Chlorpyriphos (@ 9 ml/kg) strongly suppressed the germination in both *in vitro* and pot culture, according to laboratory tests (Sithik, 2012) [9]. According to the findings of our investigation, seed treatments with test dosages of chlorpyriphos at 5, 7, and 10 ml/kg and fipronil at 7 and 10 ml/kg were harmful to maize seedlings.

The inhibition of germination and subsequent growth by pesticide treatments suggests that certain biochemical processes occurring during germination are due to rapid imbibition rate, higher seed leachate conductivity, seed respiration rate, and intensity of dehydrogenase activity that reflect the vigor of seed as affected by seed treatment, which was experimentally described by Chaudhary *et al*. (2001) [2]. According to Chopra and Chandra (1969) [3], the considerable decline in the production of reducing sugars and free amino acids in mustard was the cause of the decline in germination.

Chlorpyriphos (@ 9 ml/kg) strongly suppressed the germination in both *in vitro* and pot culture, according to laboratory tests (Sithik, 2012) [9]. The findings of our investigation showed that seed treatments with test dosages of fipronil at 7 mg/kg and chlorpyriphos at 6 mg/kg were harmful to the maize crop seedlings.

Sithik (2012) [9] examined the economics of pest management for several seed treatments using the recommended doses for a few pesticides. The most economical product was determined to be chlorpyiphos (20 EC @ 4.5 ml/kg), followed by fipronil 5SC @ 6 ml/kg (Rs. 893) and imidacloprid 17.8 SL @ 3.5 ml/kg (Rs. 1034).

Table 1: Germination percentage, seedling length and vigour indices for various seed-treatments (insecticides/termiticides only) in maize var. 'HQPM-1'

Treatments details	Germination (%)	Root length (cm)	Shoot length (cm)	Total length (cm)	Vigour Index-I	Vigour Index-II
T_1: Imidacloprid (1.5 ml/kg seed)	96.38_a [79.68]	20.70_a (4.66)	18_{cd} (4.47)	39.43_{bc} (6.34)	3927.58_{ab}(61.76)	75.05_c (8.79)
T_2: Imidacloprid (3 ml/kg seed)	95.93_a [78.88]	18.26_{cd} (4.4)	19.16_{bc} (4.60)	38.68_{bcd} (6.28)	3836.60_b (61.03)	76.74_b (8.87)
T_3: Imidacloprid (5 ml/kg seed)	94.13_b [76.23]	18.3_{cd} (4.1)	21_a (4.80)	40.13_b (6.39)	3903.18_{ab} (61.56)	79.35_a (8.02)
T_4: Fipronil (5 ml/kg seed)	94.11_b [76.22]	20.21_{abc} (4.61)	17.31_{de} (4.4)	38.83_{bcd} (6.29)	3779.11_b (60.56)	70.52_d (7.52)
T_5: Fipronil (7 ml/kg seed)	92.11_c [73.77]	20.61_{ab} (4.65)	20.2_{ab} (4.71)	42.81_a (6.59)	4069.59_a (62.87)	62.96_e (7.02)
T_6: Fipronil (10 ml/kg seed)	88.66_d [70.24]	18.45_{bcd}(4.42)	18.1_{cd} (4.48)	37.43_d (6.19)	3435.8_c (57.71)	52.68_g (6.40)
T_7: Chlorpyriphos (5 ml/kg seed)	88.11_d [69.73]	20.53_{ab} (4.65)	16.23_e (4.27)	37.76_{cd} (6.21)	3443.57_c (57.78)	56.03_f (6.62)
T_8: Chlorpyriphos (7 ml/kg seed)	82_e [66.16]	17.83_d (4.5)	13.76_f (3.97)	31.1_e (5.65)	2653.95_d (50.62)	36.90_h (5.24)
T_9: Chlorpyriphos (10 ml/kg seed)	76.33_f [62.48]	15.16_e (4.47)	9.85_g (3.44)	25.85_f (5.13)	2067.23_e (44.58)	24.00_i (4.10)
T_{10}:Control (Water)	95.88_a [78.85]	20.61_{ab} (4.65)	17.75_{de} (4.38)	38.88_{bcd} (6.29)	3854.64_b (61.16)	71.50_d (7.57)
SE(d)	0.75	0.10	0.06	0.05	0.67	0.02
CD (P=0.05)	[1.5]	(0.22)	(0.13)	(0.12)	(1.41)	(0.05)

Figures in parentheses [ ] are arcsine-transformed values and in parentheses ( ) are square root transformed values. Figures are mean of 3 replicates (each replication=100 seeds). Figures in the same column followed by the same lowercase letters are not significantly different (P=0.05)

Table 2: Effect of insecticides on seed viability

Treatment details	Maize			
	Test-dose (ml/kg)	Germn. (%)	Ungerminated seeds (%)	
			Hard seeds	Dead seeds
T_1: Imidacloprid	1.5	97	2	1
T_2: Imidacloprid	3	96	2	2
T_3: Imidacloprid	5	95	3	2
T_4: Fipronil	5	95	2	3
T_5: Fipronil	7	93	2	5
T_6: Fipronil	10	89	3	8
T_7: Chlorpyriphos	5	89	3	8
T_8: Chlorpyriphos	7	83	4	13
T_9: Chlorpyriphos	10	77	3	20

Conclusion
The most often used chemical is chlorpyriphos since it is less expensive and frequently available to farmers, however it is obvious that this chemical has severe adverse effects. Therefore, field suggestions must be made with the proper safety measures.

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