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In vitro evaluation of insecticidal seed treatments on soybean, *Glycin max* 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 (600FS), fipronil (5FS), and chlorpyriphos (20EC) (each at three dosages), were evaluated *in vitro* (lab tests) to determine whether test doses for the soybean crop had any harmful effects. Germination rates often above 80% for all treatments, including the control, much beyond the ISTA (2008) standard. However, greater chlorpyriphos dosages had a negative impact on several seedling parameters, including total seedling length and vigor indices I and II, which were measured at 26.23, 2214.4, and 36.29 (for a 6 ml/kg dose) and 20.5, 1710, and 25.86 (for an 8 ml/kg dose) correspondingly. Untreated seedlings with the equivalent control values of 35.9 cm, 3144.88 cm, and 54.86 cm are significantly higher and differ statistically (P 0.05). In the end, it was determined that seed treatment with imidacloprid at 4-6, fipronil at 3-5, and chlorpyriphos at 4 ml/kg seeds was safer and advised for soy crop. The rate of these seed treatments might be tested for additional validation under field in order to reduce pest insects that are of concern.

Keywords: *Glycin max* L, fipronil, imidacloprid, seed treatment, and chlorpyriphos

Introduction

The protection of developing seedlings from insects, diseases, and pests begins with seed treatment. Under the 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; *in vitro* tests were done on a soybean crop.

Materials and Methods

We purchased soy seeds (var. DS-9712) from the IARI's Division of Genetics in New Delhi. As per the Table 1, the seeds were given three separate dosages of each of the following: chlorpyriphos 20EC (Dursban®), fipronil 5SC (Regent®), and imidacloprid 48% FS (Gaucho® 600FS). 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 varied from 50 to 100 ml and depends 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 coating of seed. 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), 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)

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 soybean crops.

Table 1 shows the impact of three distinct pesticide dosages on soybean germination, seedling length, and vigor indicators. Germination rates often above 80% for all treatments, including the control, much beyond the ISTA (2008) standard. However, greater chlorpyriphos dosages had a negative impact on several seedling parameters, including total seedling length and vigor indices I and II, which were measured at 26.23, 2214.4, and 36.29 (for a 6 ml/kg dose) and 20.5, 1710, and 25.86 (for an 8 ml/kg dose) correspondingly. Untreated seedlings with the equivalent control values of 35.9 cm, 3144.88 cm, and 54.86 cm are significantly higher and differ statistically (P 0.05).

For imidacloprid @4ml/kg seed, the highest vigour indices (I and II) reported (3130.34, 62.84) indicated the potential for phytotonic effects on seedlings. There was an increase in the Vigour Index-II during the seed treatment of fipronil (@ 3 and 5 ml/kg), indicating a dry weight rise in the seeds while vigor index I is comparable to the values in the controls.

There is a dearth of data on various soybean growth characteristics as well as the *in vitro* testing of efficacy of seed treatments of insecticides. Narasimhulu and Kameswara Rao reported in 1989 on the impact of chemical seed treatment on the ability of germination of other oilseeds, such as peanut.

The inhibition of germination and the subsequent growth due to pesticidal treatments suggests that the certain biochemical processes occurring while germination are because of rapid rate of imbibition, higher seed leachate conductivity, rate of seed respiration, higher dehydrogenase activity that reflect the vigor of the seed as affected through seed treatment as experimentally described by Chaudhary *et al*. (2001) [1]. According to Chopra and Chandra (1969) [1], the considerable decline in the production of reducing sugars and free amino acids in mustard was the cause of the decline in germination.

Chlorpyriphos (@ 8 ml/kg) strongly suppressed germination under *in vitro* and pot culture, according to laboratory tests (Sithik, 2012) [6]. The findings of our investigation demonstrated that seed treatments with test dosages of fipronil at 7 mg/kg and chlorpyriphos at 6 mg/kg were harmful to the soybean crop seedlings.

Sithik (2012) [6] 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: Effect of seed treatment on percentage of Germination, length of seedling and vigour indices in soybean variety 'DS 9712'

Treatments details	Germination In (%)	Length of Root (cm)	Length of Shoot (cm)	Total length (cm)	Vigour Index-I	Vigour Index-II
T_1: Imidacloprid (@ 4 ml/kg seed)	83.00_{ab} [77.86]	18.01_{abc} (4.47)	17.33_{bc} (4.3)	36.38_b (6.2)	$3,130.34_b$(55.05)	62.84_c (6.34)
T_2: Imidacloprid (@ 6 ml/kg seed)	80.66_{ab} [77.45]	18.26_{abc}(4.50)	17.7_{bc} (4.34)	37.21_b (6.26)	$3,110.86_b$(54.87)	55.35_b (7.20)
T_3: Imidacloprid (@ 8 ml/kg seed)	80.16_b [75.85]	20.76_a (4.77)	19.56_a (4.54)	41.43_a (6.59)	$3,433.21_a$ (57.68)	49.05_a (7.8)
T_4: Fipronil (@ 3 ml/kg seed)	81.5_c [73.34]	19.15_{ab} (4.59)	17.31_{bc} (4.3)	36.61_b (6.21)	$3,093.77_b$ (54.71)	59.22_d (5.57)
T_5:Fipronil (@ 5 ml/kg seed)	81_d [67.65]	15.68_{cd} (4.2)	18.73_{ab} (4.50)	35.65_b (6.13)	2994.26_b (53.80)	61.04_e (5.26)
T_6: Fipronil (@ 7 ml/kg seed)	80.16_e [67.80]	11.85_e (3.71)	18.1_{abc} (4.38)	30.45_c (5.59)	$2,543.55_c$ (49.51)	57.98_f (5.19)
T_7:Chlorpyriphos (@ 4 ml/kg seed)	83.33_f [60.9]	16.65_{bcd}(4.32)	13.79_d (3.87)	31.54_c (5.79)	$2,735.56_c$ (51.39)	55.78_g (4.86)
T_8:Chlorpyriphos (@ 6 ml/kg seed)	80.66_g [52.8]	14.71_d (4.1)	10.4_e (3.42)	26.23_d (5.31)	$2,214.4_d$ (46.17)	36.29_h (3.18)
T_9: Chlorpyriphos (@ 8 ml/kg seed)	79_h [42.84]	11.7_e (3.69)	09.13_f (3.30)	20.5_e (4.68)	$1,710_e$ (40.47)	24.86_i (2.47)
T_{10}: Contrl. (Water)	84.5_a [78.31]	18.01_{abc}(4.47)	16.85_c (4.30)	35.9_b (6.16)	$3,144.88_b$ (55.18)	54.86_d (5.62)
SE (d)	0.76	0.14	0.07	0.09	1.01	0.022
CD (P=0.05)	[1.64]	(0.30)	(0.15)	(0.20)	(2.1)	(0.04)

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)

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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