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PHYTOTONIC EFFECTS OF NEONICOTINOID INSECTICIDES IN COWPEA AND GREEN GRAM

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ABSTRACT
Field experiments were conducted during *summer* and *kharif,* 2013 to study the phytotonic effects of neonicotinoid insecticides (imidacloprid, thiamethoxam and acetamiprid) as seed treatment alone and in combination with one (30 days after germination, DAG) or two foliar sprays (30 and 45 DAG) in cowpea and green gram. All the treatments of thiamethoxam (68.25 to 68.72 cm and 63.86 to 64.92 cm in cowpea (CP) and green gram (GG) respectively) significantly increased the plant height followed by imidacloprid (63.22 to 64.22 and 59.08 to 60.09 cm in CP and GG respectively) compared to untreated control. Significant increase in leaf area was also observed due to all the treatments of imidacloprid (429.27 to 431.63 and 403.61 to 406.24 cm^2 in CP and GG respectively) followed by thiamethoxam (375.43 to 381.01 and 369.46 to 372.71 cm^2 in CP and GG respectively) and acetamiprid (347.37 to 349.81 and 321.70 to 324.92 cm^2 in CP and GG respectively) compared to the untreated control. No promoting effect was observed on number of root nodules and none of the three tested insecticides had any adverse effect on seed germination of these crops.

INTRODUCTION

The effectiveness of neonicotinoid insecticides against a broad range of sucking insect pests like aphids, leaf hoppers, thrips and whiteflies have been reported in various crops like cotton (Vadodaria *et al*., 2001, Sitaramaraju *et al*., 2010, Kalyan *et al*., 2012 and Bharpoda *et al*., 2014), okra (Praveen, 2005), cowpea (Patel *et al*., 2012), green gram (Nakat *et al*., 2002, Khutwad *et al*., 2002) etc. Imidacloprid, thiamethoxam and acetamiprid are the three major neonicotinoid insecticides which are used against these pests. In addition to its effectiveness in pest management, these insecticides as seed treatment and foliar spray have been reported to exert growth promoting effects (phytotonic effects) such as increase in leaf area (Sreelatha and Divakar, 1997), plant height (Sitaramaraju *et al*., 2010), chlorophyll content (Preetha and Stanley, 2012), root growth (Macedo *et al*., 2013) and number and weight of root nodules (Singh *et al*., 2000) in various crops. An ideal insecticide used for seed treatment should not exert any adverse effects on seed germination and hence, the effect of neonicotinoid insecticides on seed germination needs to be investigated. Moreover, the applied insecticide must not have any deleterious effects on root nodules of legume crops.

Cowpea, *Vigna unguiculata* (L.) Walpers and green gram, *Vigna radiata* (L.) Wilczek are two important pulse crops grown in India during both *kharif* and *summer* season. Neonicotinoid insecticides *viz*., imidacloprid, thiamethoxam and acetamiprid as seed treatment and in combination with foliar sprays can be an effective option for the control of both early season and late season sucking insect pests of cowpea and green gram such as aphid, leaf hopper, and whitefly. However, the information about the phytotonic effects of neonicotinoid insecticides on these crops and their effects on seed germination is absent. With this background, the experiments were conducted to study the phytotonic effects of these three neonicotinoid insecticides as seed treatment alone and in combination with foliar spray in cowpea and green gram and the effects of seed treatment with neonicotinoid insecticides on seed germination of cowpea and green gram.

MATERIALS AND METHODS

In order to assess the phytotonic effects of neonicotinoid insecticides in cowpea (variety: Gujarat cowpea -1) and green gram (variety: Meha), field experiments were conducted in randomized block design during *summer* and *kharif,* 2013 in the agronomy farm, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat with ten treatments and three replications. Five plants were selected randomly from each net plot and various growth parameters such as leaf area (at 65 DAG), plant height (at 65 DAG) and root nodules (at 50 DAG) were recorded. Based on these observations, mean leaf area (cm^2), mean plant height (cm) and mean number of root nodules per plant were calculated. Leaf area was measured by leaf area meter. The procedure proposed by Chander and Singh, 1993 was adopted with slight modifications.

The treatments evaluated were

T_1 : Seed treatment (ST) with imidacloprid 600 FS @ 5 ml/ Kg seed *i.e.,* 3 g a.i./kg seed

T_2 : T_1 + Foliar spray of imidacloprid 17.8 SL (0.008 % - 40 g a.i./ha) at 30 days after germination (DAG)

T_3 : T_1 + Foliar spray of imidacloprid 17.8 SL (0.008 %- 40 g a.i./ha) at 30 and 45 DAG

T_4 : ST with thiamethoxam 35 FS @ 5 ml/kg seed *i.e.* 1.5 g a.i./Kg seed

T_5 : T_4 + Foliar spray of thiamethoxam 25 WG (0.01 % - 50 g a.i./ha) at 30 DAG

T_6 : T_4 + Foliar spray of thiamethoxam 25 WG (0.01 % - 50 g a.i./ha) at 30 and 45 DAG

T_7 : ST with acetamiprid 20 SP @ 20 g/kg seed *i.e.*, 4 g a.i./Kg seed

T_8 : T_7 + Foliar spray of acetamiprid 20 SP (0.01% - 50 g a.i./ha) at 30 DAG

T_9 : T_7 + Foliar spray of acetamiprid 20 SP (0.01% - 50 g a.i./ha) at 30 and 45 DAG

T_{10} : Untreated Control (Water spray)

Recommended dosages of fertilizers were applied and other agronomic practices were done. In order to determine the effect of neonicotinoid insecticides on seed germination, two separate laboratory trials with cowpea and green gram seeds were conducted. The experiments were laid out in completely randomized design with four treatments (imidacloprid 600 FS @5ml/Kg seed, thiamethoxam 35 FS@5ml/Kg seed, acetamiprid 20 SP @ 20 g/Kg seed and untreated control) and five repetitions for each treatment. Hundred seeds each of cowpea and green gram treated with respective neonicotinoid insecticide and untreated seeds were placed evenly in germination paper rolls (rolled paper towel method) and kept in the germinator at 25ºC. Number of germinated seeds were recorded after 7 days when majority of the seeds germinated. Based on these observations, germination percentage was calculated.

The data on plant height, leaf area and root nodules were statistically analyzed by ANOVA technique and the data on seed germination was also analysed by ANOVA technique after applying arc sine transformation (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Plant height

Data (Table 1) on plant height of cowpea recorded during *summer* showed that significantly more plant height (67.31 to 68.81 cm) was recorded in all the treatments of thiamethoxam *i.e.* sole treatment (ST alone) and combination treatments (ST + one or two foliar sprays at 30 or 30 and 45 DAG) as compared to the sole treatment and combination treatments of acetamiprid (54.62 to 56.10 cm) and the untreated control (49.60 cm). The treatments (ST alone and in combination with foliar spray) of imidacloprid also significantly increased the plant height of cowpea (63.63 to 64.60 cm) and were found at par with the treatments of thiamethoxam. The data revealed that the treatments of acetamiprid comprising of ST alone and in combination with one or two foliar sprays improved the plant height, but failed to exert their significant effects. The results obtained during *kharif* followed quite similar trend as observed during *summer* and all the treatments of thiamethoxam recorded significantly higher plant height followed by the treatments of imidacloprid. Pooled over seasons data also showed that the ST with thiamethoxam alone and in combination with foliar sprays of thiamethoxam significantly increased the plant height (68.25 to 68.72 cm) followed by the sole treatment and combination treatments of imidacloprid (63.22 to 64.22) cm.

The experimental results (Table 1) on plant height followed the similar trend in green gram during *summer* and *kharif* season as noticed in cowpea. Superiority of all the treatments of thiamethoxam followed by imidacloprid in improving the plant height was revealed during both *summer* and *kharif* season. It was also observed that all the treatments of acetamiprid could not improve significantly the plant height of green gram, although it had remarkably increased the plant height. Pooled over seasons data clearly indicated that thiamethoxam was superior to the treatments of imidacloprid and acetamiprid in enhancing the plant height and the treatments of acetamiprid did not significantly increase the plant height.

It was also inferred that there were no significant differences in plant height in cowpea and green gram between the sole treatment (seed treatment alone) and the combination treatments (seed treatment combined one or two foliar sprays) of any particular insecticide during both the seasons.

Increase in plant height of cotton due to ST with imidacloprid and thiamethoxam has been documented by earlier researchers (Vadodaria *et al.*, 2001., Prasanna *et al.*, 2004 and Sitaramaraju *et al.*, 2010). However, Sitaramaraju *et al.* (2010) reported that maximum plant height was recorded in seed treatment with imidacloprid than thiamethoxam which contradicts with the present findings on the superiority of thiamethoxam in increasing the plant height than imidacloprid. From the available source of information, it could be revealed that the effects of seed treatment with acetamiprid on plant height has not been reported by any researchers and hence cannot be compared. Preetha and Stanley (2012) found that imidacloprid, thiamethoxam and acetamiprid as foliar spray exerted a significant increase in the plant height of cotton and okra, with maximum increase in thiamethoxam treated plants which also corroborates with the present results.

Leaf area

The data on mean leaf area of cowpea (Table 2) showed that significantly higher leaf area (432.73 to 439.03 cm^2) was found in the treatments of imidacloprid (seed treatment alone and in combination with foliar spray(s)) followed by the treatments of thiamethoxam (382.73 to 389.87 cm^2) during *summer*. All the three treatments of acetamiprid also improved the leaf area (350.32 to 356.03 cm^2) but was at par with the untreated control (306.87 cm^2). Quite similar results were found during *kharif* season as it was noticed in *summer*, wherein the plots treated with imidacloprid showed significantly greater leaf area (420.37 to 430.53 cm^2) in comparison to acetamiprid which was at par with the untreated control. Thiamethoxam treatments were found mediocre in its phytotonic effect and the recorded leaf area ranged from 368.13 to 378.14 cm^2. Pooled over seasons data revealed that ST alone with imidacloprid and ST with imidacloprid + single (30 DAG) or double (at 30 and 45 DAG) sprays showed significantly higher leaf area (429.27 to 431.63 cm^2) than rest of the treatments. Thiamethoxam and acetamiprid applied as seed treatment

Table 1: Effect of neonicotinoid insecticides on plant height of cowpea and green gram during 2013

Treatments	Mean plant height (cm) at 65 DAG					
	Cowpea			Green gram		
	Summer	Kharif	Pooled	Summer	Kharif	Pooled
T_1	64.60	63.85	64.22	57.52	60.65	59.08
T_2	64.30	63.74	64.02	58.82	61.36	60.09
T_3	63.63	62.81	63.22	57.03	61.53	59.28
T_4	67.31	69.19	68.25	63.71	66.13	64.92
T_5	68.81	68.62	68.72	64.52	65.10	64.81
T_6	67.35	69.28	68.32	63.51	64.21	63.86
T_7	55.86	54.85	55.36	50.58	51.01	50.80
T_8	54.62	53.93	54.28	51.63	50.45	51.04
T_9	56.10	54.52	55.31	52.01	49.73	50.87
T_{10}	49.60	47.39	48.50	45.74	43.83	44.78
S Em ±						
T (Treatment)	3.75	4.29	2.56	3.52	3.46	2.20
S (Season)	-	-	1.27	-	-	1.09
T x S	-	-	4.03	-	-	3.44
C D at 5 %						
T	11.14	12.74	7.31	10.46	10.27	6.27
S	-	-	3.74	-	-	3.21
T x S	-	-	NS	-		NS
C V (%)	10.61	12.21	11.43	10.79	10.43	10.59

NS: Non-significant

Table 2: Effect of neonicotinoid insecticides on leaf area of cowpea and green gram during 2013

Treatments	Mean leaf area (cm^2) at 65 DAG					
	Cowpea			Green gram		
	Summer	*Kharif*	Pooled	*Summer*	*Kharif*	Pooled
T_1	434.51	424.03	429.27	389.67	417.55	403.61
T_2	432.73	430.53	431.63	392.33	420.15	406.24
T_3	439.03	420.37	429.70	395.00	415.59	405.30
T_4	382.73	368.13	375.43	358.92	380.00	369.46
T_5	383.87	378.14	381.01	363.13	382.29	372.71
T_6	389.87	372.03	380.95	366.48	378.37	372.42
T_7	351.90	342.84	347.37	319.74	323.65	321.70
T_8	350.32	349.30	349.81	320.08	324.74	322.41
T_9	356.03	340.23	348.13	313.38	336.46	324.92
T_{10}	306.87	290.40	298.64	265.49	287.99	276.74
S Em ±						
T (Treatment)	24.07	23.67	15.15	23.37	25.25	15.49
S (Season)	-	-	7.55	-	-	7.69
T x S	-	-	23.87	-	-	24.30
C D at 5 %						
T	71.51	70.34	43.19	69.42	75.03	44.15
S	-	-	22.21	-	-	22.62
T x S	-	-	NS	-	-	NS
C V (%)	10.89	11.03	10.96	11.62	11.93	11.78

NS: Non-significant

alone and in combination with single (30 DAG) or double (at 30 and 45 DAG) sprays were found at par and were superior to untreated control.

Superiority of imidacloprid followed by thiamethoxam in increasing the leaf area observed in cowpea was also revealed in green gram (Table 2). During *summer*, significantly higher leaf area (389.67 to 395.00 cm^2) was registered in imidacloprid treatments than the untreated check and the acetamiprid treatments. Similar trend of treatment effect was observed during *kharif* season. With respect to the phytotonic effect in increasing the leaf area, imidacloprid stood first in its rank followed by thiamethoxam and acetamiprid. Minimum phytotonic effect with respect to leaf area was observed in acetamiprid which was at par with the untreated check. Pooled over seasons data indicated that all the insecticidal treatments registered significantly higher leaf area than the untreated check. The treatments of imidacloprid exhibited significantly higher leaf area followed by the treatments of thiamethoxam and acetamiprid.

There were no significant differences in leaf area of cowpea and green gram between the sole treatments (seed treatment alone) and the combination treatments (seed treatment combined one or two foliar sprays) of a particular insecticide during *summer* and *kharif* season.

Increase in leaf area would result in increased photosynthetic efficiency and thus increased yields. The present findings is in

Table 3: Effect of neonicotinoid insecticides on root nodules in cowpea and green gram during 2013

Treatments	Mean no. of root nodules/plant at 50 DAG					
	Cowpea			Green gram		
	Summer	*Kharif*	Pooled	*Summer*	*Kharif*	Pooled
T_1	7.97	7.35	7.66	6.91	7.68	7.30
T_2	8.52	7.04	7.78	7.10	6.98	7.04
T_3	7.36	7.82	7.59	6.70	7.33	7.02
T_4	8.39	7.44	7.91	7.65	7.39	7.52
T_5	7.83	8.37	8.10	7.08	7.11	7.10
T_6	7.72	7.53	7.62	6.78	7.58	7.18
T_7	7.87	7.61	7.74	7.29	7.18	7.24
T_8	8.17	8.40	8.28	7.51	8.07	7.79
T_9	6.98	7.79	7.38	6.92	6.90	6.91
T_{10}	7.28	7.52	7.40	7.34	7.14	7.24
S Em ±						
T (Treatment)	0.58	0.51	0.17	0.61	0.47	0.18
S (Season)	-	-	0.38	-	-	0.39
T x S	-	-	0.54	-	-	0.57
C D at 5 %						
T	NS	NS	NS	NS	NS	NS
S	-	-	NS	-	-	NS
T x S	-	-	NS	-	-	NS
C V (%)	12.58	11.53	12.17	14.88	11.14	12.98

NS: Non-significant

Table 4: Effect of neonicotinoid insecticides as seed treatment on seed germination of cowpea and green gram during 2013

Treatments	Germination (%)					
	Cowpea			Green gram		
	Summer	*Kharif*	Pooled	*Summer*	*Kharif*	Pooled
Imidacloprid(5 ml/kg seed)	*74.31(92.68)	69.61(87.86)	71.96(90.41)	71.81(90.25)	73.23(91.67)	72.52(90.98)
Thiamethoxam(5 ml/kg seed)	72.19(90.64)	71.80(90.24)	72.00(90.45)	74.03(92.43)	72.22(90.67)	73.12(91.57)
Acetamiprid(20 g/kg seed)	73.21(91.65)	69.98(88.28)	71.60(90.03)	72.96(91.41)	74.53(92.88)	73.74(92.16)
Untreated control	72.77(91.22)	71.34(89.76)	72.05(90.50)	72.24(90.69)	74.33(92.70)	73.28(91.72)
S Em ±						
T(Treatment)	1.53	1.76	0.92	2.09	1.73	1.18
S (Season)	-	-	0.46	-	-	0.57
T x S	-	-	0.92	-	-	1.14
C D at 5 %						
T	NS	NS	NS	NS	NS	NS
S	-	-	1.38	-	-	NS
T x S	-	-	NS	-	-	NS
C V (%)	3.42	3.80	2.87	4.48	3.77	3.48

NS: Non-significant; *Figures are arc sine transformed values whereas those in parentheses are re-transformed values

conformity with the results of Sreelatha and Divakar (1997) who reported that ST with imidacloprid significantly increased the leaf area of okra. However, Macedo *et al*. (2013) reported that there was a slight decrease in leaf area of the forage grass, *Brachiaria* with the increase in the doses of thiamethoxam. However, from the available source of information, it could be found that none of the earlier researchers had investigated the promoting effects of acetamiprid on the leaf area of crops.

Root nodules

The data (Table 3) on mean number of root nodules per plant at 50 DAG obtained during *summer* and *kharif* season showed non-significant differences among the different evaluated treatments in both the seasons and crops. Non-significant results were also obtained when the pooled data over seasons were statistically analysed. It indicates that the evaluated neonicotinoid insecticides (imidacloprid, thiamethoxam acetamiprid) did neither exert any promoting effect nor had any adverse effect on nodulation of cowpea and green gram. On the other hand, Iliewa and Vasilewa (2012) reported that seed treatment with imidacloprid increased the root nodulation in soybean. Similarly, Singh *et al*., 2000 reported that seed treatment with thiamethoxam increased the number and weight of nodules per plant in soybean which does not support the present findings, as it was found that none of the tested neonicotinoid insecticides had any promoting effects on the root nodules of cowpea and green gram. Similarly, Seidenglanz *et al*. (2010) also reported that application of thiamethoxam significantly increased the root nodulation in field pea (*Pisum sativum* L.)

Effect of neonicotinoid insecticides on seed germination

Data (Table 4) on the effect of neonicotinoid insecticides on seed germination indicated non-significant differences among different treatments in both cowpea and green gram during *summer* and *kharif* season. The pooled over seasons data also showed non-significant results. The experimental data implies that ST with imidacloprid 600 FS (5 ml/Kg seed),

thiamethoxam 35 FS (5 ml/Kg seed) and acetamiprid 20 SP (20 g/Kg seed) had no adverse effect on the germination of cowpea and green gram seeds. These insecticides can be safely used for the management of sucking insect pests. Effect of neonicotinoid insecticides (imidacloprid and thiamethoxam) as seed treatment on seed germination of various crops such as cotton (Prasanna *et al*., 2004), okra (Praveen, 2005) and sunflower (Mutkule *et al.,* 2010 and Sajjan *et al.,* 2010) have been studied by earlier researchers in past. The present results are in conformity with their findings wherein it was reported that there was no adverse effect due to seed treatment with these insecticides on seed germination of these crops. The present study however, is not in conformity with the findings of Almeida *et al*. (2014) who reported that seed treatment with thiamethoxam increased the seed germination of common bean, (*Phaseolus vulgaris* L.). Similarly, Dutta *et al*. (2015) reported that seed priming with captan + imidacloprid enhanced the seed germination in chilli and the disagreement in the results with the present study may be due to the differences between the procedures for seed priming and seed treatment and may be also due to the combined effect of captan and imidacloprid.

On the other hand, Palumbo and Sanchez (1995) found that in greenhouse cage studies, whitefly, *Bemisia tabaci* Gennadius developed very high densities of nymphs and eclosed pupal cases on musk melon plants not treated with imidacloprid and the significant increase in vegetative plant growth was inversely proportional to whitefly densities. However, positive plant growth responses were not observed when plants were treated with imidacloprid and the insects were excluded. Hence, they concluded that growth and yield response to imidacloprid is associated with control of whiteflies and the subsequent prevention of damage, rather than a compensatory physiological promotion of plant growth processes. On the other hand, Macedo and e Castro (2011) found that thiamethoxam as seed treatment influenced the early growth of wheat plants by increasing root development, altered the distribution of photoassimilates, increased the concentration of total soluble protein, reduced the nitrate reductase activity and increased the phenylalanine ammonia-lyase activity to a certain dose and also increased the ear dry weight and number of fertile tillers. These reports highlights the importance of more further studies on the biochemical and the physiological basis of the growth promoting effects shown by the neonicotinoid insecticides.

Thus, it can be concluded that the neonicotinoid insecticides (imidacloprid, acetamiprid and thiamethoxam) as seed treatment alone and in combination with foliar spray exert significant growth promoting effects in cowpea and green gram by enhancing the leaf area and plant height, in addition to controlling the sucking pests and thereby may result in increased yields. These insecticides also do not exert any adverse effect on seed germination.

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