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Efficacy of pesticide seed treatments against wheat aphid and its effect on coccinellid predator and crop yield

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Abstract
Field experiment was conducted to evaluate the effect of two neonicotinoid insecticides alone and in combination with fungicide (Tebuconazole) as a seed dressing for the control of aphids and their effect on coccinellids and yield of two wheat cultivars (ARRI-2011, Millat-2011). Results showed that at each observation date staring from January to March, significantly more aphids were present in the plots having control treatment compared to the plots where crop was grown with pesticides treated seed. Imidacloprid and actara alone and in combination with tebuconazole showed non-significant difference from one another in term of management of aphids at each observation date on both varieties. Results also showed that the plots having seed treated with imidacloprid and thiamethoxam have less number of coccinellids compared to those which received imidacloprid and thiamethoxam mixture with tebuconazole at each observation date. Significant more germination of both varieties treated with Hombre (267.67, 272.12 per m^{-2}) and actara + tebuconazole (256.00, 260.44 per m^{-2}) was observed compared to the seeds treated with imidacloprid, actara and control. Seed dressing with Hombre and actara + tebuconazole significantly increases the grains per spike and 1000 grain weight of two cultivars of wheat sown during 2015-2016. The strategy of using Hombre and actara + tebuconazole combinations as a seed treatment allowed easy application of pesticides, gave satisfactory and reliable control of wheat aphids and found to be safer to non-target organisms compared to the foliar application of pesticides.

Keywords: seed treatment, wheat aphid, neonicotinoids, tebuconazole, coccinellids, grain yield

1. Introduction
Wheat (*Triticum aestivum* L.) is one of the world's most important cereal crop and is grown in all provinces of Pakistan, with the largest area under cultivation. It plays very significant role in the economic stability of the country [12]. Despite the huge cultivation area in the country, wheat production doesn't meet the annual demand due to comparatively low production per hectare. This is due to several abiotic and biotic factors, such as outdated approaches of cultivation, lack of insect pest and disease resistant varieties, lack of irrigation facilities, rain-fed dry farming, low soil fertility level and attack of insect pests and diseases on standing crop [9].

In Pakistan, wheat crop is attacked by several insect pests such as army worm, cut worm (Noctuidae Lepidoptera), termites (Isoptera: Termitidae), wheat beetles and weevils (Coleoptera: Chrysomelidae, Carabidae, Curculionidae) and Aphids (Aphididae: Homoptera) [24]. However aphids cause severe damage to wheat crop as compared to all other insect pests. Direct yield losses to wheat crop due to sucking of cell sap are 30-45% while indirect losses are 20-80% as they transmit several fungal and viral diseases [7, 12, 14, 18]. Adults and nymphs of aphids reduced the vigor of plants at several stages by sucking the cell sap. Sometimes due to the toxicity of the saliva of some aphid species young shoots were found killed. Honey dew excretion is often prolific and sooty moulds usually accompany aphid's infestation which eventually affects the rate of photosynthesis in plants. Aphids multiply very rapidly under favorable conditions on leaves, stems and inflorescence of crop [10, 21].

The application of pesticides as seed dressing is safest, cheapest and effective means of controlling most seed and soil borne pathogens. Currently in Pakistan, use of fungicides as seed treatment is the most widely followed practice in wheat. All commercial seed of cultivated wheat is treated with fungicides to enhance germination of seed and seedling stand, to reduce the damage caused by root rot, seed rot and other soil born fungi. Seed dressing with fungicides also helps to minimize yield loss or to maintain and improve quality and to avoid further spread of pathogens [13]. However, Insecticidal seed treatment has not been followed to control the sucking insect pests (aphids) of this crop.

Mostly, the aphid populations remain below the economic injury level depending upon the environmental condition and can be easily controlled combination with naturally occurring population of predators on this crop. But sometimes, the aphids become extremely injurious if present in large number and chemicals are applied to control infestation. It can easily be controlled by foliar application of insecticides [6, 20], but being a staple food, wheat should not be treated with foliar application of insecticides due to the environmental concerns and other economic and technical reasons. Insecticides with low mammalian toxicity can be applied as seed dressing to control aphids. Previous studies also showed that the insecticide treated seed has antifeedant effects against wheat aphid at low rate [2]. There are several studies which showed that a different mixture of neonicotinoid insecticides and fungicides are very helpful to control diseases and sucking insect pests of wheat [2, 22, 23]. No local data are available for management of aphids through the application of different insecticide mixture with fungicides as seed dressing. So the current study was designed to evaluate the effect of two neonicotinoid insecticides alone and with a fungicide mixture as seed dressings for the management of aphids to reduce reported yield losses. Moreover, the response of these treatments was also evaluated against coccinellid (natural predator of aphid) and wheat crop yield under field conditions.

2. Materials and Methods

Field experiment was conducted at the farm area of Adaptive Research Farm, Risala #5 Sargodha, Pakistan during crop season 2015-2016. Two wheat cultivars (AARI-2011 and Millat 2011) were obtained from the Punjab Seed Corporation. Sowing of crop was done in lines on 15th November, 2015. For each cultivar, a total area of 2023 m^2 was divided into three blocks and each block was separated by 0.8 m distance. The experiment was replicated three times by using Randomized Complete Block Design (RCBD).

Following treatments were used in the experiment at recommended doses.

T1: Hombre (Imidacloprid + Tebuconazole) @ 4 ml/kg seed

T2: Actara + Tebuconazole @ 0.6 g + 1.57 ml/kg seed

T3: Imidacloprid @ 2.5 g/kg seed

T4: Actara (Thiamethoxam) @ 0.6 g/kg seed

T5: Control

After application of pre-sowing irrigation, when soil moisture reached at the field capacity level field was prepared by using tractor mounted cultivator followed by planking. Treated seeds were sown by hand drill using recommended seed rate (50 kg/acre). Standard agronomic practices were applied uniformly for wheat crop sown in each block. Urea, diammonium phosphate (DAP) and Sulfate of Potash (SOP) were used as a source of nitrogen, phosphorus and potash respectively. Crop was irrigated three times with canal water until maturity.

2.1 Data Collection

The population of aphid and coccinellid was recorded at 15 days interval starting from January (15-01-2016) to March (30-03-2016). There were six readings of aphid and coccinellid population on Jan 15, Jan 30, Feb 15, Feb 29, March 15 and March 30 of 2016. During the sampling of aphids three areas were selected randomly from each block and from which six plants were selected to count aphids. The number of aphids per tiller of each plant was recorded as aphid density. For the population of coccinellids, three areas of one square meter were selected from each block and coccinellids were counted per spike after counting the number of tillers.

The crop was harvested at maturity on 12th April. Other parameters studied were germination count/m^2, root length, shoot length, number of grain/ spike, 1000-grain weight, grain yield.

2.2 Statistical Analysis

The obtained data were analyzed by using Statistix 8.1(Analytical software, Statistix; Tallahassee, Florida, USA, 1985-2005) following Randomized Complete Block Design. Means were separated by using the Tukey HSD test at the 5% level of significance.

3. Results

3.1 Effect of seed treatments on aphid and coccinellid population

The effect of two neonicotinoids insecticides alone and with a fungicide mixture on aphid population (aphid/tiller) is shown in Fig.1 (A, B). Results of ANOVA revealed that the maximum population of aphid was observed on 15th of March in both varieties. However, cultivar of wheat did not affect the population of aphids and there was no significant difference between population of aphids on both the varieties on each observation date ($p<0.05$). At every date of observation, control treatment showed a significantly higher aphid population as compared to all other treatments ($p<0.05$). Imidacloprid and thiamethoxam alone and in combination with tebuconazole showed non-significant difference from one another in term of management of aphids at each observation date on both varieties. When crops reach near to maturity the population of aphids automatically reduced in each treatment including control.

Effect of pesticide treatment on coccinellid predator is shown in Fig. 2 (A, B). There was no coccinellid observed during first two observation date (15- January and 30-January) on both the varieties. Population of coccinellids appeared on both varieties after 30th of January and compared to treated one, high population was observed afterwards in control plots. Maximum coccinellid population was observed on 15th of March on ARRI-2011 (2.76/plant) while on Millat -2011 population was 3.0/plant on 30th of March and it was non-significantly different from the population observed on 29th February (2.53/plant) and 15th of March (2.70/plant). Results showed that the plots having seed treated with imidacloprid and Acara had less number of coccinellid compared to the population observed in plots those received imidacloprid and thiamethoxam mixture with tebuconazole at each observational date Fig. 2 (A, B).

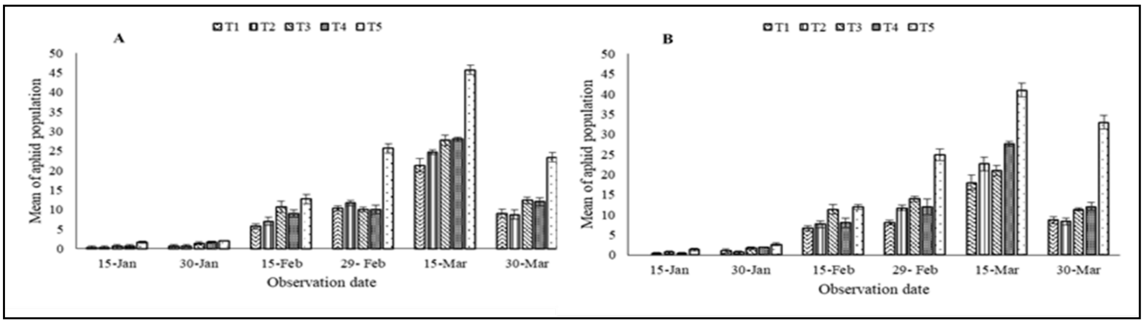

Fig 1: Mean number of aphids per spike on ARRI (A) and Millat (B) cultivars of wheat during 2015-2016 season

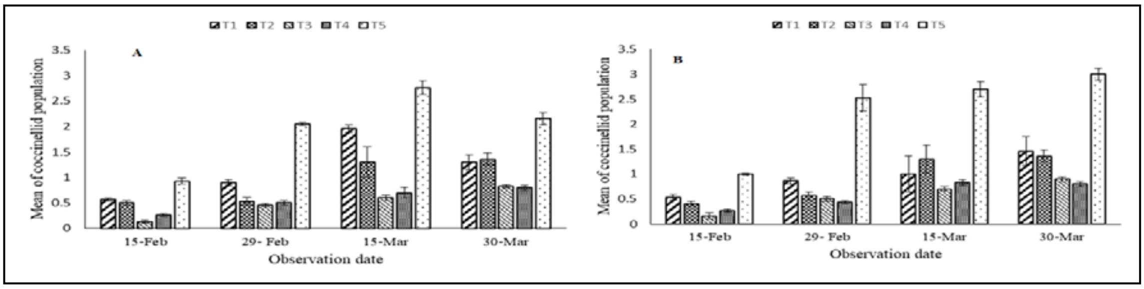

Fig 2: Mean number of Coccinellid per spike on ARRI (A) and Millat (B) cultivars of wheat during 2015-2016 season

3.2 Effect of seed treatments on growth and yield parameters of two wheat cultivars

Analysis of variance showed that there was a significant difference in all treatments on yield and growth parameters of wheat varieties. However, both varieties did not differ in terms of 1000 grain weight and grains per spike (Table 1).

Table 1: ANOVA of different growth and yield parameters of two wheat varieties sown during 2015-2016 season.

Source of variation	Germination/m^2		Grains/spike		1000 grain weight		Root length		Shoot length		Yield	
	MS	F	MS	F	MS	F	MS	F	MS	F	MS	F
Treatment	953.16**	32.06	550.41**	175.66	155.18**	118.15	39.87**	167.69	283.54**	189.28	2152011**	254.93
Variety	136.53*	4.59	7.50.0^{ns}	2.39	5.15^{ns}	3.91	2.25*	9.50	7.17*	12.40	190488**	22.57
Treatment × Variety	0.20^{ns}	0.01	6.58^{ns}	2.10	0.034^{ns}	0.30	0.02^{ns}	0.01	0.16^{ns}	0.29	1280^{ns}	0.15
Error	29.73	---	3.13	---	1.31	---	0.23	---	0.57	---	8441	---

Observation of growth and yield parameters of two cultivars of wheat under field conditions. ns = non-significant (p>0.05); * = significant (p<0.05); ** = highly significant (p<0.01).

There was non-significant interaction effect between varieties and treatments (Treatment × Variety) regarding all parameters of growth and yield of both varieties. Germination of the crop was observed after two weeks of sowing and results showed that there was significantly more germination of seeds of both varieties treated with Hombre (267.67, 272.12 per m^{-2}) and actara + tebuconazole (256.00, 260.44 per m^{-2}) compared to seeds treated with imidacloprid and actara alone. There was non-significant difference between T1 and T2. Similarly T3 and T4 were also non-significantly different from one another. However, all treatments were significantly different from control (T5) (Table 2 A). Similar trend was observed for root and shoot length. More shoot and root length was observed for Hombre and actara + tebuconazole treated seeds compared to seed treated with imidacloprid, actara and control treatment.

Table 2(B) showed that seed dressing with Hombre and actara + tebuconazole significantly increased grains per spike and 1000 grain weight of two cultivars of wheat sown during 2015-2016 season. Both treatments (T1 and T2) appeared non-significantly different from one another. Highest yield was observed in T1 while the yield observed in T2 was non-significantly different from T3. There was also a significant increase in grains per spike, 1000 grain weight and yield of AARI-2011 and Millat-2011 after treatment with imidacloprid and actara compared to the control treatment. Least number of grains per spike, 1000 grain weight and yield were observed in the control treatment.

Table 2: Effect of different pesticides as seed treatment on growth (A) and yield (B) parameters of two wheat varieties sown during 2015-2016 season. (A)

Treatments	Germination/m^2		Root length (cm)		Shoot length (cm)	
	ARRI-2011	Millat-2011	ARRI-2011	Millat-2011	ARRI-2011	Millat-2011
Hombre (T_1)	267.67 ± 1.45^{A}	272.12 ± 1.57^{A}	13.14 ± 0.40^{A}	13.80 ± 0.42^{A}	27.13 ± 0.59^{A}	28.49 ± 0.62^{A}
Actara + Tebuconazole (T_2)	256.00 ± 4.51^{AB}	260.44 ±3.31^{AB}	12.89 ± 0.09^{A}	13.53 ± 0.10^{A}	24.03 ± 0.54^{B}	25.23 ± 0.57^{B}
Imidacloprid (T_3)	249.67 ± 0.88^{B}	254.32 ± 3.21^{B}	11.03 ± 0.08^{B}	11.58 ± 0.09^{B}	18.50 ± 0.33^{C}	19.42 ± 0.35^{C}
Actara (T_4)	245.34 ± 2.19^{BC}	249.63 ±1.65^{BC}	10.69 ± 0.35^{B}	11.23 ± 0.37^{B}	18.00 ± 0.28^{C}	19.35 ± 0.30^{C}
Control (T_5)	234.00 ± 4.58^{C}	237.41 ± 1.96^{C}	6.87 ±.26^{C}	7.21 ± 0.27^{C}	9.56 ± 0.26^{D}	10.04 ± 0.27^{D}

Means sharing same letters in each column are statistically non-significant (p>0.05).

(B)

Treatments	Grains/spike		1000 grain weight (g)		Yield (kg ha^{-1})	
	ARRI-2011	Millat-2011	ARRI-2011	Millat-2011	ARRI-2011	Millat-2011
Hombre (T_1)	48.33 ± 0.88^A	50.67 ± 2.19^A	31.56 ± 0.82^A	33.75 ± 0.85^A	3833.80 ± 33.30^A	4025.50 ± 35.00^A
Actara + Tebuconazole (T_2)	47.00 ± 0.58^A	50.00 ± 1.00^A	32.76 ± 0.28^A	32.51 ± 0.29^A	3447.70 ± 74.40^B	3620.10 ± 78.10^B
Imidacloprid (T_3)	40.33 ± 0.67^B	42.56 ± 0.67^B	27.77 ± 0.32^B	28.60 ± 0.39^B	3321.50 ± 67.10^B	3487.60 ± 70.50^B
Actara (T_4)	39.34 ± 1.20^B	39.00 ± 0.56^B	25.70 ± 0.70^B	26.47 ± 0.72^B	306.80 ± 33.40^C	3220.1 ± 35.10^C
Control (T_5)	26.67 ± 0.88^C	24.67 ± 0.33^C	20.20 ± 0.85^C	20.80 ± 0.87^C	2266.90 ± 33.41^D	2380.20 ± 35.10^D

Means sharing same letters in each column are statistically non-significant ($p>0.05$).

4. Discussion

Aphids are major pests of wheat under field conditions and can cause severe losses to grain yield if proper management practices are not followed. In the current study, seed of two cultivars (ARRI-2011, Millat-2011) of wheat was treated with two neonicotinoid insecticides alone and with fungicide mixture. Neonicotinoids are relatively safer compared to the other insecticides. Their seed treatment has important advantages over a conventional spray treatment as it reduces hazards and has a longer period of protection on the irrigated crop [2]. Results showed that there was less population of aphids in the blocks where neonicotinoids alone or in combination with fungicides were used as a seed treatment. There was no consistent performance of all treatments (T1-T4) throughout the season and population of aphid's increased significantly during February and March in all treatments. However this was less than the control treatment. Present results are in line with previous studies. Burd *et al*. (1996) [4] reported acute toxicity to aphids fed on plants grown from seeds treated with imidacloprid, which protected plants for 45 days after sowing, while Ahmed *et al* (2001) [2] reported that imidacloprid in combination with tebuconazole can control aphids for 8 weeks when applied as a seed treatment. Our results are also in agreement with Suhail *et al*., 2013[22]. They found that aphid population was lowest on those plants of wheat which were grown with seed treated with Hombre and actara. Similarly, Royer *et al*., (2005) [20] found that seed treatment with imidacloprid and other insecticides decreased the population of sucking insect pests such as cereal aphids and leaf hoppers. Liu *et al*. (2005) [15] and Abd-Ella *et al*. (2016) [1] found that imidacloprid and thiamethoxam seed treatments can efficiently control wheat aphids throughout the wheat growing season, and increased wheat production. Imidacloprid and thiamethoxam have a similar mode of action and molecular structures, and they interact likewise with the nicotinic acetylcholine receptors (nAChRs) of the central and peripheral nervous systems [1, 25]. Population of coccinellid was low in the crop which was grown with seed treated with imidacloprid and thiamethoxam alone compared to their mixture with fungicides. Our results are in agreement with Suhail *et al*., 2013[22]. They found that insecticide mixture with fungicides were more toxic to coccinellids compared to fungicides alone. Different insecticides have different toxicity to the coccinellids. Moser and Obrycki, 2009 [17] also found that neonicotinoid have neurotoxic effects on larvae of coccinellids when exposed to seedlings grown from the seeds treated with these insecticides. Several studies show that when coccinellids were exposed to pesticide residue present in the nectar of flowers, or when fed on pesticides contaminated food, it altered its feeding behavior, growth and reproduction. Plant products are supplement of their diet and can create a potential route of exposure to systemic insecticides used as seed treatments and seed treatments can produce sublethal impacts on the biology of coccinellid predators [16].

Application of insecticide and fungicide mixture increases the growth and yield parameters of both cultivars of wheat, this is in accordance with several previous studies [3]. Increase in germination was resulted due to control of seed rot and pre emergence damping off diseases as reported by Ahmed *et al*. (1994) [3]. Fungicide mixture with insecticide as also increased the shoot and root length which is in accordance with Pike *et al*., 1993; Kaspers *et al*., 1987; Holderness, 1990 [8, 11, 19]. They stated that pesticide mixture at low dose stimulates the growth of roots and shoots. Germination, root and shoot length in seeds treated with imidacloprid and actara were more as compared to the control treatment is due to induction of salicylic acid-associated responses which also elicits plant protection and helps in abiotic stress [5]. Low germination in control is due to soil fungi which can contaminate seed and can affect growth and germination of plants. Similarly 1000 grain weight, grain per spike and yield was also more in crop which was grown from seeds treated with the mixture of insecticide and fungicides [2, 22].

5. Conclusion

Results of current study suggest that imidacloprid and thiamethoxam mixture with tebuconazole seed treatment can be used to avoid the risk of a delayed spray, reduce early aphid infestation and increase crop yield. However these results needs further research on determination of sublethal effects of insecticides on natural enemies, such as coccinellids which are major predators of aphids on wheat. These results also need research studies in view of the extended period of systemic activity of neonicotinoid insecticides in wheat aphids.

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