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Full Length Article

Efficacy of Different Neem (*Azadirachta indica*) Products in Comparison with Imidacloprid against English Grain Aphid (*Sitobion avenae*) on Wheat

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Abstract

Different neem (*Azadirachta indica* L.) extracts (leaf and seed) and seed oils were tested against English grain aphid (*Sitobion avenae* F.) with a neonicotinoid, imidacloprid on wheat. All neem oils and neem seed kernel extract proved comparatively more effective in laboratory bioassay studies, with LC_{50} values ranging from 0.34 to 1.10%. In field-cage experiment, neem seed oil-hexane was statistically similar with imidacloprid followed by neem seed oil-expeller and neem seed kernel extract, while neem seed oil-ethanol was at par with imidacloprid. On the basis of cumulative insect days in field trials, neem seed oil-expeller proved as effective as imidacloprid in controlling the aphids, while except neem seed cake extract, all other treatments were statistically at par with imidacloprid. Non-significant differences were found in the population of mummified aphids between control and neem seed kernel extract, neem leaf extract, neem seed oil-hexane, neem seed cake extract and neem seed oil-expeller. There existed non-significant differences in all treatments regarding aphid predators. Maximum increase in wheat yield (7.28 q/ha) was observed in neem seed kernel extract treated plots followed by those of imidacloprid (7.23 q/ha). Application of imidacloprid resulted in maximum cost-benefit ratio (1:1.34) followed by that of neem seek kernel extract 5% (1:1.31). If the additional cost of loss of beneficial organisms and environmental risks posed by the application of synthetic insecticides is considered, the cost-benefit ratio of neem seed kernel extract may be comparable to that of imidacloprid and may be used against wheat aphids.

Keywords: Wheat; *Sitobion avenae*; Neem leaf extract; Neem seed extract; Neem oil; Imidacloprid

Introduction

Wheat is the peoples staple food in Pakistan; which ranks 8th largest wheat producer in the world and accounts for 2,09,58,000 MT production on an area of 8549.9 thousand hectares (GOP, 2008). Wheat crop is affected both directly and indirectly with infestation of aphids. The direct damage is caused by sucking cell sap from the tender leaves and young shoots resulting in distortion, stunting, leaf curling and wilting. Indirect damage occurs through the transmission of plant viruses and development of sooty mould on honey dews secreted by aphids (Rossing *et al.*, 1994; Akhtar and Mujahid, 2006; Zeb *et al.*, 2011). Aphids can cause 35- 40% yield losses directly (Kiechefer and Gellener, 1992) and 20-80% losses by transmitting viral and fungal diseases (Trdan and Mileroj, 1999). Due to certain climatic changes, aphid population has been increasing for the last few years on wheat crop and has attained the status of pest in all wheat growing areas of Pakistan (Aheer *et al.*, 2008). English grain aphid (*Sitobion avenae* F.) is one of the most dominant aphids of wheat in many countries of the world including subcontinent. It causes damage by infesting upper mature leaves, spikes and heads of the plants (Tradan and Mileroj, 1999). Maximum loss in yield occurs in case of infestation between ear emergence and flowering which may reach up to 14% (Liu *et al.*, 1986).

Control of aphids with synthetic insecticides is not desirable on wheat due to many bad effects like pesticide residues, destruction of predators and parasites, environmental pollution, destabilization of ecosystem and enhanced resistance to insecticides in pests. So, there is a need to explore eco-friendly and cost-effective control methods. Among available non-chemical weapons, neem (*Azadirachta indica*) has the potential to be used as a substitute of synthetic insecticides (Schmutterer, 1995; Farooq *et al.*, 2011; Basedow *et al.*, 2002; Rashid *et al.*, 2012). Products derived from seeds, leaves, kernels and other parts of the neem tree are inexpensive and biodegradable naturally available source (Shafeek *et al.*, 2004) and have been found to be effective against different pests (Gahukar, 2000; Liang *et al.*, 2003; Senthil Nathan *et al.*, 2005). According to Schmutterer and Singh (2002), more than 540 insect species including key insect pests of agricultural and horticultural crops have been found to be susceptible to azadirachtin and exhibited various behavioral and physiological effects (Satti *et al.*, 2010).

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Present study was conducted with the aim to find out the efficacy of three different neem extracts and their results were compared with imidacloprid (the most widely used insecticide worldwide against sucking insects including aphids) in laboratory, semi field and field conditions to calculate their cost-benefit ratio. So that the farmers may adopt environment friendly and cost effective practices for the management of cereal aphid.

Materials and Methods

Neem Aqueous Extracts

To make neem leaf extract (NLE), one kg of green neem leaves were mixed with water to make 5 L of the solution to get 20% of NLE. The leaves were soaked overnight in water. The next day, they were ground and extract was filtered. To the filtrate, an emulsifier like Khadi soap (soap with no detergent) was added @ 1 mL in 1 L water. In order to make neem seed kernel extract (NSKE), outer coat of neem kernel was removed and pounded (hammered) gently, in such a way that no oil came out. Fifty grams of neem kernel powder (between three and eight months old) was gathered in muslin pouch and soaked overnight in water to make up the volume to 1 L (5%). The pouch was squeezed and the extract was filtered and khadi soap (soap with no detergent) was added at the rate of 1 mL/L water as an emulsifier One hundred grams of neem cake was added in water to make volume 1 L of solution (10%) to prepare neem cake extract (NSCE) The neem cake was put in a muslin pouch and soaked in water overnight. It was then filtered and an emulsifier was added at the rate of 1mL for 1 L of water (Musabyimana *et al*., 2001).

Neem Oils

Neem seed oil-ethanol (NSO-ethanol) was prepared by grinding two hundred grams de-coated neem seeds in a blender to make seed powder, which was added to ethanol to make up to 1 L volume and kept for a week. The solution was filtered through a filter paper and evaporated with the help of soxhlets apparatus to get solvent free oil. The oil was weighed to calculate the concentration of oil and solution of desired concentration was made. For making neem seed oil-hexane (NSO-hexane), same method was followed as described above, only hexane was used in place of ethanol for extracting neem oil (Charmaine *et al.*, 2005). Neem oil extracted by expeller (NSO-expeller) was purchased from the local market. The concentration of oil was calculated (by taking information from the shopkeeper) and solution of desired concentration was made (Musabyimana *et al.*, 2001).

Imidacloprid

Noenicotinoid insecticide, imidacloprid (Confidor 25 WG) was used as a standard to compare of the results of different neem products against wheat aphids, which is considered most widely used insecticide against sucking pests including aphids (Caroline, 2001).

Toxicological Studies of Different Neem Extracts, Oils and Imidacloprid

Toxicological studies of different neem products were conducted in the laboratory under control conditions (25±2ºC and 75±5% RH). Seeds of wheat (variety Wafaq) were sown in small pots (15 cm diameter) in field conditions, containing top layer fertile soil, sand and well rotten farm yard manure in 1:1:1 ratio. After 21 days of germination, thinning was done so that each pot contained only three seedlings. These pots were shifted into the laboratory. For each product, a stock solution of desired concentration was prepared with water and subjected to seven serial dilutions. On each pot, ten female aphids (4^{th} instar) were released on wheat seedlings and allowed to settle down. Each pot was covered with round plastic sheet having muslin cloth on its top. Next day these aphids were counted again and dead ones were replaced with new healthy aphids. These pots were sprayed thoroughly with each test product with the help of hand sprayer and covered again with round plastic sheets having muslin cloth. Each concentration was comprised of four replicates and forty aphids. Four pots were sprayed with only water in each bioassay to serve as a control. The data regarding mortality of aphids were taken 3 days after application of insecticides. All the data were processed through probit analysis using Polo-PC software (Russel *et al*., 1977).

Testing of Different Neem Extracts, Oils and Imidacloprid in Semi Field Conditions

Toxicological studies of different neem products were conducted in the semi natural condition. Wheat plants were sown in earthen pots in field conditions. After 35 days of germination thinning was done so that each pot contained only five tillers. These pots were kept in iron cages covered with muslin cloth. On each pot twenty five 4^{th} instar female aphids (*Sitobion avenae* F.) were released on wheat plants and allowed to settle down. These pots were covered with iron cages containing muslin cloth. Next day these aphids were counted again. Most of the aphids were found settled while dead aphids were replaced the new healthy ones. Then these pots were sprayed thoroughly with insecticide preparation with the help of calibrated sprayer and covered again. Each treatment was replicate four times. Four pots were sprayed with water only to serve as a control. The data regarding mortality of aphids were taken 3, 5 and 7 days after application. All the data was subjected to analysis of variance (ANOVA) and the means were compared by using Duncan's Multiple Range test at 5% level of probability (Steel *et al*., 1996).

Testing of Different Neem Extracts, Oils and Imidacloprid in Field Conditions

The experiment was laid out in Completely Randomized Block Design with eight treatments and three replicates. Wheat crop was sown on 04-11-2010, with 4.5× 9 m plot size. These plots were separated from each other by keeping distance of 0.6 m between each treatment and 1ft between each replicate. All agronomic practices were applied in all experimental plots according to the recommended rates. The plots were observed continuously for the infestation of cereal aphids and application of neem products was done when their population reached 10 aphids per tiller. Application was mad with calibrated knapsack sprayer (Gardena, Model Art 884) during morning hours.

The data regarding wheat aphids and their natural enemies were taken one day before application and 4, 7 and 11 days after application. Further application was not needed because the aphid's population became low in all the experimental plots including control. In each experimental plot nine tillers were clipped randomly following zigzag pattern and shifted in plastic zip bags. These bags were kept in ice box and shifted to laboratory. The data regarding aphids, mummified aphids, lady bird beetles, syrphid fly larvae and green lace wing larvae were taken by counting their number from the whole tillers. The data were converted to cumulative insect days to get an estimate of both intensity and duration of *S. avenae* and its natural enemies. An insect day may be defined as one insect feeding on one plant for 24 h. Insect days for a sampling date were calculated with the formula adapted from Ruppel (1983) as $a_d = ([X_d + X_{d-1}]/[2]t$, where a_d = number of insect days between the current sampling date and previous sampling date. X_d = Number of insects per plant on the current sampling date. X_{d-1}= Number of insects per plant on previous sampling date, and t = the number of days between sampling dates. Cumulative insect days were calculated by summing the number of insect days for each sampling date. Yield of wheat was taken by randomly selecting three spots of 1 m^2 from each experimental plot. The plants were cut, shifted into plastic bags and brought to the lab. Wheat grains were threshed manually after drying, weighed with electric balance and average yield/m^2 was found out for each plot.

Statistical Analysis

All the data was subjected to ANOVA and the means were compared by using Duncan's Multiple Range test at 5% level of probability (Steel *et al*., 1996).

Results

Bioassay Studies

In the lab bioassay, NLE and NSCE were found least effective against English Grain aphid with comparatively more LC_{50} values than the other neem preparations. NSKE was the most with 1.10% LC_{50} value. All the three neem oils proved more effective with comparatively less LC_{50} values, and NSO-n-hexane appeared most toxic than the other two, with 0.34% LC_{50} value. The synthetic insecticide imidacloprid proved the most toxic (LC_{50}= 2.85 ppm) than all the neem products (Table 1).

Semi Field

Data regarding mortality (%) of English grain aphid showed significant differences among all test formulations, 3, 5 and 7days after application of different neem products revealed. Maximum aphid mortality was observed in plants treated with imidacloprid and NSO-n-hexane followed by NSO-ethanol, NSO-expeller and NSKE treated plants (Table 2). All the neem oils performed better than neem extracts except NSKE. Minimum mortality was observed in case of NLE and NSCE.

Field

All the neem treatments significantly reduced the pest population. Imidacloprid and NSO-Expeller gave the lowest cumulative insect days, while all other neem treatments except NSCE were at par with imidacloprid, showing effectiveness of all neem oils and two extracts. Highest cumulative insect days of mummified aphids were in found in control. Non significant differences were found between control and NSKE, NSCE, and NSO-n-hexane treated plots. Imidacloprid exerted relatively more adverse impact on parasitized aphids with lowest cumulative insect days than all other treatments. The comparison of cumulative insect days showed no significant difference among all treatments in case of aphid predators (lady bird beetles, syrphid fly and green lacewing). All the neem preparations reduced the cereal aphid population in field trials, but their impact on aphid parasitoids was low, in contrast to imidacloprid treated plots.

Cost-benefit Ratio

The CBR of different neem preparations and imidacloprid was calculated to find out the best treatment with maximum control of the pest and yield with a minimum cost. The maximum increase in the yield was observed to be 7.28 q/ha in those plots, where NSKE (5%) was applied followed by imidacloprid (7.23 q/ha). The results pertaining to the wheat yield and cost benefit ratio in different methods of control are depicted in Fig. 1. The application of NSKE (5%) resulted in a CBR of 1:1.31 and was found to be the most economical as compared to that of all the other neem preparations followed by NSO-Expeller 0.5% (1:1.17) and NLE 20% (1:1.15). Application of imidacloprid (neonicotinoid synthetic insecticide) resulted in maximum CBR i.e., 1:1:34.

Table 1: Toxicity of different neem formulations and imidacloprid against English grain aphid (*Sitobion avenae*) after 72 h

Insecticide	LC_{50} (95%CL)	Fit of probit line			
		Slope(±SE)	χ^2	df	Heterogeneity
Neem Leaf Extract %	14.75(8.92-32.49)	1.00±0.18	0.38	5	0.08
Neem Seed Kernal Extract%	1.10(0.73-1.71)	1.05±0.16	0.11	5	0.02
Neem Seed Cake Extract %	19.29(8.19-144.30)	0.74±0.184	0.12	5	0.02
Neem Seed Oil (Ehanol) %	0.67(0.44-0.99)	1.29±0.20	1.16	5	0.23
Neem Seed Oil (n -Hexane) %	0.34(0.23-0.51)	1.46±0.16	1.17	5	1.17
Neem Seed Oil (Expeller) %	0.67(0.46-1.04)	1.10±0.16	1.73	5	0.35
Imidacloprid (Confidor) ppm	2.85(1.78-4.11)	1.29±0.17	1.40	5	0.28

Table 2: Means comparison of mortality (%) of English grain aphid (*Sitobion avenae*) after application of different neem formulations and imidacloprid in semi-field conditions

Treatment	*Sitobion avenae*		
	3DAT	5DAT	7DAT
Neem Leaf Extract (%)	26.0±2.58 d	39.0±2.52d	46.0± 2.58c
Neem Seed Kernel Extract (%)	75.0±2.52 a	79.0±1.63 c	89.0±1.91 b
Neem Seed Cake Extract (%)	16.0± 2.31e	33.0±1.91 d	46.0±2.58 c
Neem Seed Oil-Ethanol (%)	62.0± 2.58bc	86.0±2.58 b	96.0± 1.63ab
Neem Seed Oil -n-Hexane (%)	66.0±2.58 b	92.0± 2.83ab	100.0±0.00 a
Neem Seed Oil –Expeller (%)	58.0± 2.00c	79.0±3.00 c	89.0±6.19 b
Imidacloprid (ppm)	77.0± 1.91a	95.0±1.00 a	100.0± 0.00a
Control	5.0± 1.00f	7.0±1.00 e	9.0±1.00 d
DMR (0.05)	6.55	6.39	7.92
MS	3169.64	4734.21	4595.93
CV	9.33	6.64	7.55

Means sharing similar letters are not significantly different (P>0.05)
DAT = Days after treatment

Table 3: Cumulative insect-days (per plant) of English grain aphid (*Sitobion avenae*) and its natural enemies after application of different neem formulations and imidacloprid in field experiments

Treatment	Aphids	Mummified Aphids	Lady bird Beetles	Syrphid fly Larvae	Green Lace Wing larvae
Neem Leaf Extract	60.91±3.37bc	3.25±0.32ab	1.00±0.61a	0.148±0.07a	0.278±0.18a
Neem Seed Kernel Extract	63.30±1.91bc	4.17± 0.47a	1.09± 0.47a	0.130±0.13a	0.203±0.20a
Neem Seed Cake Extract	72.59±8.86b	4.09± 0.28a	0.54±0.16a	0.074±0.07a	0.000±0.00a
Neem Seed Oil (Ethanol)	63.28±2.25bc	2.37± 0.46b	0.0±0.00a	0.074±0.07a	0.000±0.00a
Neem Seed Oil (n-Hexane)	58.28±3.58bc	4.00±0.48a	0.54±0.15a	0.074±0.07a	0.074±0.07a
Neem Seed Oil (Expeller)	56.28±6.66c	3.30± 0.26ab	0.0±0.00a	0.000±0.00a	0.000±0.00a
Imidacloprid	53.37±2.59c	0.94±0.38c	0.46±0.36a	0.000±0.00a	0.000±0.00a
Control	92.48± 4.76a	4.19±0.19a	0.74±0.44a	0.000±0.00a	0.000±0.00a
CD (P=0.05)	14.41	1.11	1.04	0.209	0.298

Means sharing similar letters are not significantly different (P>0.05)

Discussion

The results of toxicological studies support the effectiveness of NSKE and all the neem seed oils against English grain aphid. Neem oils and neem seed extracts based formulations have been reported to give significant reduction against green peach aphid on pepper, current lettuce aphid on lettuce and strawberry aphids on strawberry with LC_{50} values ranging from 0.2 to 1.4% (Lowery *et al.*, 1993). In the present studies, NSO-hexane proved better insecticide than NSO-ethanol and NSO-expeller with lowLC_{50} values. Similar trend was observed with the application of different neem preparations against *Lipaphis erysimi*, where neem seed oil extracted in hexane exhibited much higher insecticidal activity the neem seed aquous and ethanolic extracts of neem (Singh *et al.*, 1988). Khalequzzaman and Nahar (2008) found that azadirachtin was more toxic than

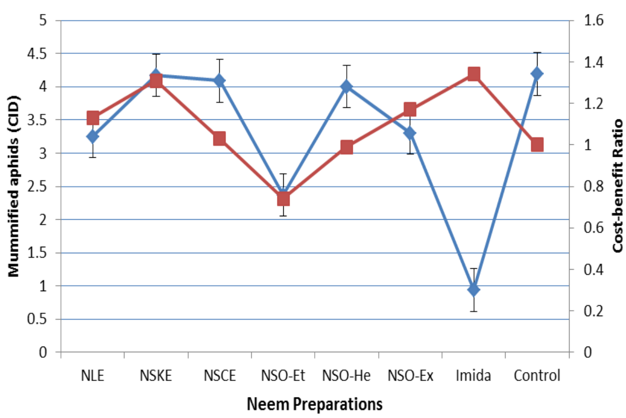

Fig. 1: Comparison of mummified aphids (cummulative insect days) with cost-benefit ratio of different neem products against English grain aphid (*Sitobion avenae*)

imidacloprid, malathion, carbosulfan and cymbush to control four aphid species namely *Aphis craccivora*, *A. gossypii*, *Myzus persicae* and *Lipaphis erysimi*.

The results of field cage experiments revealed varied response of neem water extracts and oils against wheat aphids. NSO-hexane and NSO-ethanol performed better than NSO-expeller and produced statistically similar results to imidacloprid. These differential effects of neem products against test aphid species may be attributed to the differences in the methods of extraction influencing the bioactivity of the neem formulations. Neem oils may help to spread the chemicals on both plant and animal surface and allow them to penetrate into the insects, therefore the products containing both azadirachtin and oils may exhibit better aphicidal activities than either ingredients alone (Stark and Walter, 1995). Previous studies advocate the effectiveness of neem formulations against different aphids in the semi field conditions. Application of AZ (180 ppm) on potted citrus plants reduced 100% citrus aphids 7 days after treatment (Tang *et al*., 2002). Neem azal T/S was most effective along with Decis and Karate against cereal aphids, *Rhopalosiphum padi* and *Metopolophium dirhodum* on wheat plants in green house conditions (Sallam *et al.*, 2009). Spray of neem extract caused more than 95% mortality of *R. padi* on barley (Sengonca and Brüggen, 1991). On the basis of cumulative insect days, except NSCE all the neem preparations produced comparable results to imidacloprid, under field conditions. The difference in the bio-efficacy of NLE field and lab studies may be due to the variation in the environmental conditions of these experiments as suggested by Lowery *et al.* (1993). Moreover pungent smell may also be the reason for low aphid population of NLE treated plots. Lowery *et al*. (1993) reported that NSO and NSE treatments were as effective as the botanical pyrtherum for control of aphids on pepper and strawberry. According to Melesse and Singh (2012), NSKE proved better than all other treatments against pea aphid, *Acyrthosiphon pisum* and can be used for the management of pea aphid. Application of 5% NSKE controlled the mustard aphid, *Lipaphis erysimi* to 13 aphids per plant on treated plots than 40 aphids per plant on untreated plots (Sanjeev and Singh, 2008).

The population of mummified aphids was very low in imidacloprid treated plots and maximum, in plots treated as a control. The comparison of the cumulative insect days reflected no or low (as in NSO-ethanol treatment) significant negative impact on aphid parasitism in plots treated with neem as compared to control. In an earlier study, Tang *et al.* (2002) found similar emergence of parasitoids from neem seed extract (Neemix) treated and untreated citrus brown aphids and concluded that application neem extract had a little impact on the survival and development of aphid parasitoids. There was no significant difference in the cumulative insect days in plots treated with neem as compared to control for lady bird beetles, syrphid fly larvae and green lace wing larvae. Many previous findings support the safety of neem based insecticide formulations towards predators. Eisnenlohr *et al*. (1992) reported that the number of syrphid fly larvae in the field was not reduced after spraying of neem Azal-F on peach trees infested by *Myzus persicae.* Similarly Neemix was found to have little or no impact on lady bird beetles, parasitic wasps, spiders and predatory mites (Hoelmer *et al.*, 1990; Stark *et al.*, 1990). Kaethner (1991) used AZT-VR-K (1000 ppm) and a mixture of this product with NO (250–3000 ppm) on broad spectrum predator *Chrysoperla carnea i*n the laboratory and semi-field trials. The products had no toxicity on eggs, larvae, adults and fecundity of the preadator. Similarly, Vogt (1993) was also unable to find the significant influence of neem-Azal-F on larvae of the lacewing in field trials. The neem products must be ingested to be effective, therefore insects, which feed on plant tissues, are affected by the extract and those which feed on other insects rarely contact lethal concentrations, which may lead to their insensitivity to the neem extracts. The compatibility of neem formulations with beneficial insects of wheat is especially important to use them in conjunction with parasitoids and predators for management of wheat aphids. Maximum increase in the yield was observed NSKE (5%) treated plots followed by imidacloprid. Whereas application of imidacloprid resulted in maximum CBR (1:1:34) followed by that of NSKE 5% (1:1.31), which reflects that imidacloprid is cheap as compared to neem extract. But if the additional cost of loss of beneficial insects and environmental risks posed by the application of insecticides is considered, the CBR of neem seed kernel extract may be regarded comparable to that of imidacloprid.

In conclusion, the effectiveness of both neem seed extracts and oils against English grain aphid. In field trials NSO-expeller proved as effective as imidacloprid against wheat aphids. Wheat yield was maximum in NSKE treated plots, which in-turn yielded highest CBR among all the neem preparations. Although CBR of NSKE was less as compared to imidacloprid, yet keeping in mind safety towards natural enemies and environment it may considered as a promising substitute of synthetic insecticides to control wheat aphids.

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