Research Article

Assessment of the Lethal and Parasitism Effects of *Helicoverpa armigera* Nucleopolyhedrovirus (HaNPV) on *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae)

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Abstract | Studies were conducted in the laboratory to assess the effects of *Helicoverpa armigera* nucleopolyhedrovirus (HaNPV) applied to eggs of *Sitotroga cerealella* Olive, on emergence of and parasitism by female *Trichogramma chilonis* (Ishii) emerged from HaNPV- treated host eggs. The percent emergence (mean) of tiny parasitoid from host eggs treated during egg, larval and pupal stages with the virus at field dose (x), 2x dose, and 0.5x dose ranged from 83.2- 87.3%, 87.1- 91.6%, and 83.7-89.7%, respectively, relative to control eggs treated with water. The parasitism rates by female parasitic wasps emerged from host eggs treated at x dose when parasitoid was during egg, larval, and pupal stages were 21.6, 26.5, and 25.1%, respectively; the 2x dose resulted in parasitism (mean) of 23.1, 27.6, and 26.1 when parasitoids were treated in egg, larval, and pupal stages, respectively, while, 0.5x dose led to 21.0, 25.9, 27.7, mean parasitism, respectively. The percent reduction in both emergence (mean) and parasitism (mean) relative to the controls was less than 10% for all doses and life stages treated, indicated that HaNPV is harmless (E< 30%) to the emergence as well as parasitism efficiency of *T. chilonis* emerged from treated host eggs.

Editor | Tahir Sarwar, The University of Agriculture, Peshawar, Pakistan
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Citation | Khan, M. A., Khan, H., Farid, A. 2014. Assessment of the lethal and parasitism effects of *Heicoverpa armigera* Nucleopolyhedrovirus (HaNPV) on *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae). *Sarhad Journal of Agriculture*, 30(4): 425-432.
Keywords | *Trichogramma chilonis*, Parasitism, HaNPV, Control.

Introduction

Trichogramma wasps are widely distributed in the world and play key roles as biological control agents of pests belonging to the order Lepidoptera in a variety of agricultural crops (Hassan and Abdelgader, 2001). Several species of parasitic wasps in the genus *Trichogramma* are reared and released for controlling insect pests of corn, rice, cotton, sugar-beet, tomatoes, vegetables, and orchard crops (Hassan, 1993; Smith, 1996) in an estimated 50 nations worldwide.

Understanding pesticide effects on beneficial insects is an integral part of rational pesticide use in integrated pest management (Croft, 1990; Johnson and Tabashnik, 1999). Such knowledge is helpful in devising and adopting strategies that minimize the adverse effects of pesticides such as use of selective compounds, and altered dosage or schedule of pesticide applications (Way, 1986; Hassan et al., 1994; Martinson et al., 2001). The high mortality and other negative effects of the non-selective, broad spectrum pesticides on performance of beneficial arthropods are commonly understood and interfere with effective IPM (Ruberson et al., 1998). Therefore, characterizing both lethal and sublethal effects is necessary for effectively integrating chemical and biological control (Stark et al., 2007), as it is important to select pesticides that have minimal lethal and sublethal impacts on natural enemies (Desneux et al., 2006), but also provide effective

suppression of the target pest.

Nucleopolyhedroviruses (NPV) belong to the major group of arthropod viruses known as baculoviruses. NPVs are widely used as biocontrol agents of insect pests (Moscardi, 1999), and have been used since the early 1890s (Huber, 1986). They are obligate pathogens and replicate in the host cells. They usually infect their larval hosts following ingestion (Andreadis, 1987), although vertical transmission and injection by parasitoids (Harper, 1986) are known. They are generally host specific and have been found in seven insect orders and in certain Crustacea (Federici, 1997). They are commonly associated with the Lepidoptera and Hymenoptera (Mazzone, 1985).

Helicoverpa armigera nuclear polyhedrosis virus (HaNPV) is a microbial pesticide, is commercially available as "Helicovex"in Pakistan, and is effective for managing lepidopteran pests, including, *Helicoverpa armigera* (Hübner) in cotton, tomato, pea, tobacco, maize, sweet corn and lettuce. *H. armigera* larvae also attack pulses, sunflower, wheat, lucerne, potato and other crops in Pakistan (Ahmed et al., 1992).

Trichogramma chilonis (Ishii) is widely distributed throughout the Indian subcontinent and has been used to successfully manage lepidopteran pests in various agro-ecosystems (Manjunath et al., 1985). Some of the common hosts in Pakistan include sugarcane borer (*Chilo sacchariphagus*) in sugar cane, diamondback moth (*Plutella xylostella*) in cabbage and other vegetables, and cotton bollworms (*Helicoverpa armigera*) in cotton and corn. *Helicoverpa armigera* is successfully managed by *T. chilonis* in cotton (Rasool et al., 2002).

It is, therefore, necessary to assess compatibility of this minute wasp with the use of pesticides, including biopesticides, under the umbrella of IPM. This study assessed the effect of HaNPV on parasitoid emergence from treated host's eggs and subsequent parasitism of freshly exposed untreated eggs by the females of *T. chilonis* emerged from HaNPV- treated eggs, in order to determine the compatibility of the said microbial pesticide with use of the parasitoid to manage *H. armigera* in agroecosystem.

Materials and Methods

Studies were conducted in the laboratory of the Entomology Division in Nuclear Institute of Food and Agriculture (NIFA), Tarnab, Peshawar during 2011 to evaluate effects of HaNPV on the emergence of *T. chilonis* from treated hosts eggs, and also on the parasitism efficacy of female parasitoid emerged.

Rearing of *Sitotroga cerealella* Olive on wheat grain

Sitotroga cerealella Olive adults were collected daily from infested grains into oviposition jars (10 cm x 15 cm, having mesh number "35- 40" fixed to their bottoms) using an electric suction apparatus. The collection jars were placed on a tray containing starch for oviposition. The moths laid eggs on the starch, and eggs were collected daily using sieves (mesh no. 50, 70). The collected host eggs were spread on sterilized wheat grain in a plastic jar (14 x 22 cm) for larval development and subsequent adult emergence. The host eggs were used both to provide hosts for rearing and experiments with *T. chilonis,* and to maintain *S. cerealella* in the laboratory at 24 ± 6°, 60 ±10% RH, and 16:8 h (L: D).

Rearing of *T. chilonis*

The grain moth eggs (approximately 800-1000) were sprinkled on glued cards (4 cm x 7 cm), followed by subsequent glue drying, and cards were exposed to parasitoids in glass jars (5 cm x 12 cm) containing approximately 20-30 pairs of adult of *T. chilonis* in the laboratory at conditions described earlier. After 24-h of exposure, the parasitized cards were moved to another glass jar of the same size and incubated at 23 ± 3°, 70 ±10% RH and 16:8 h (L:D), until adult emergence.

Preparation of different concentrations of HaNPV solutions

Formulated HaNPV available in the market contains 7.5 x 1012 OBs of HaNPV/L, and the recommended field rate is 80ml/acre. This rate was serially diluted in tap water to prepare three concentrations, i.e., field recommended concentration (FRC: x), twice field rate (2x), and half than field rate (0.5x)

Testing to evaluate effects of the HaNPV on emergence and subsequent parasitism by the emerged female

Ten parasitized cards, each containing approximately 10 to 15 moth eggs (1 x 4 cm), were used for each stage-specific trial. Cards containing eggs that had been exposed to parasitoids one (egg test), three (larval test), and seven days (pupal test) after parasit-

ism were dipped individually in FRC, 2x, and 0.5x of HaNPV, and tap water (untreated control) for 1-2 seconds, and subsequently removed and dried at room temperature for at least 1 h. After drying, they were placed in glass vials (1 cm x 7 cm). The vials containing parasitized eggs cards were incubated under the same conditions described earlier (until the first adult emerged).

Individual cards contained 250 to 300 fresh eggs of *Sitotroga* were exposed after first parasitoid adult emerged in each vial for 24 hours at laboratory conditions for completion of parasitism and all the females emerged (ranged 5 - 8) during first 24 h have parasitized the newly exposed cards. After 24 hours, exposed egg cards were removed from each vial and placed in separate vials and incubated under the stated conditions until pupal formation (after 7 days).

Emergence data was recorded by counting all the emerged adults found dead in each vial, while parasitism data was recorded by counting the total parasitized eggs in each vial 7 days after exposure, and the number of parasitizing females which emerged during the first 24 hours when egg cards were exposed were also recorded in order to estimate mean parasitism per emerged female *T. chilonis*. Data was recorded separately for each stage.

Statistical analysis

The data was transformed (square root transformed) prior to analysis of variance (split-plot design). Statistical software "Statistix 9", the Tukey's post hoc HSD ($p = 0.05$ or 5%, all pair wise comparison) test was used for separation and comparison of means. The reduction in emergence and parasitism is calculated with formula (1-T/C)*100, where T equals percent emergence (mean) in treated and C is percent emergence (mean) in control.

The toxicity is ranked under IOBC/WPRS (IOBC/WPRS, 1994): Class: 1, harmless (E < 30%); 2, slightly harmful (30% ≤ E ≤ 79%); 3, moderately harmful (80% ≤ E ≤ 99%); 4, harmful (E > 99%). "E" stand for pesticides effect (%) on either emergence or parasitism

Results and Discussion

The result of ANOVA (Table 1) for percent emergence (mean) of *T. chilonis* indicated significant dif

Table 1. *Analysis of variance for emergence of T. chilonis based on square root transformed data regarding stage and dose wise treatment with HaNPV*

Source	DF	SS	MS	F	P
Replication	9	100.21	11.135	-	-
Stage	2	430.08	215.038	25.44	0.000
Error Rep*Stage	18	152.15	8.453	-	-
Dose	3	212.40	70.798	6.01	0.001
Stage*Dose	6	117.57	19.595	1.66	0.141
Error rep*stage*Doses	81	954.61	11.785	-	-
Total	119	1967.02	-	-	-

Table 2. *Analysis of variance for parasitism by T. chilonis based on square root transformed data regarding stage and dose wise treatment with HaNPV*

Source	DF	SS	MS	F	P
Replication	9	5.433	0.604	-	-
Stage	2	566.742	283.371	403.05	0.0000
Error Rep*stage	18	12.655	0.703	-	-
Dose	3	57.531	19.177	12.02	0.0000
Stage*Dose	6	49.433	8.239	5.17	0.0002
Error Rep*Stage*Dose	81	129.191	1.595	-	-
Total	119	820.985	-	-	-

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ferences (Tukey HSD, $p \leq 0.05$) both for dose and life stage, but the interaction between them was not significant, with $p = 0.141$ ($p > 0.05$). Similarly, the ANOVA results (Table 2) demonstrated significant differences for parasitism per parasitoid female in response to both, life stage at time of treatment and dose, but also showed significant ($p \leq 0.05$) interaction between stage and dose.

The cumulative percent emergence (mean) of all doses in addition to control of each parasitoid life stage differed significantly among the immature stages (Table 3, $p \leq 0.05$). However, the three doses tested against each stage of minute parasitoid demonstrated no significance difference with each other and with their respective control treatments regarding emergence and were statistically at par ($p > 0.05$).

The percent reduction in emergence over control (Table 4) revealed that reduction (%) in emergence in response to the treated dose were not significantly different from each other in the treatment of larvae and pupae stages ($p > 0.05$). While, 2x and x doses of egg treatment showed significant difference ($p \leq 0.05$). The cumulative mean reduction in emergence for all treated doses of each stage demonstrated significance difference between egg and larval stage treatment. Similarly, the cumulative mean value for each dose based on all treated stages also showed significance difference between x and 2x doses ($p \leq 0.05$).

Both 2x and x doses of HaNPV (Table 5) tested against egg stage of minute parasitoid demonstrated no significance difference with each other and with their respective control treatments regarding parasitism by the female emerged ($p > 0.05$). All the treated doses in larval treatment regarding parasitism by the female *T. chilonis* emerged demonstrated statistically at par with each other and with their respective control treatment (Table 5, $p > 0.05$). Similarly, 2x and 0.5x doses were also statistically at par with each other and with their respective control treatment regarding parasitism by the female emerged from host eggs treated when parasitoid was at pupal stage ($p > 0.05$). The cumulative mean parasitism based on all doses for larvae and pupae stages of parasitic wasp were not significantly different with each other ($p > 0.05$), while mean dose response based on all treated stage for parasitism demonstrated significance difference between x and 2x doses ($p \leq 0.05$).

The percent reduction in parasitism over control (Table 6) revealed that 2x dose of egg treatment was significantly different from the 0.5x and x doses ($p \leq 0.05$). Similarly, in the larval and pupal treatments, 2x dose were also found significantly different from 0.5x dose ($p \leq 0.05$).

The cumulative mean reduction in parasitism for all treated doses of each stage demonstrated statistically at par with each other ($p > 0.05$). Similarly, the cumulative mean value for each dose based on all treated stages also showed no significance difference between 0.5x and x doses ($p > 0.05$).

Both emergence (mean) and parasitism (mean) relative to the control treatment (Figure 1) in response to HaNPV were greater than 90% at all doses and for all immature stages, in other words, the percent reductions both in emergence and parasitism relative to the control (Table 4, 6, respectively) revealed that all three stages and doses demonstrated less than 10% reduction in emergence and parasitism, which indicates that HaNPV is harmless ($E < 30\%$) to emergence of as well as parasitism by *T. chilonis* regarding all the doses used and all the stages treated.

The use of biological control is increasing: based on 1) greater awareness of environmental and food safety concerns created by the application of traditional chemicals in agro-ecosystems, and 2) the failure of conventional pesticides due to development of resistance in insect pests (Dent, 1993). Therefore, such chemicals are increasingly replaced with the most compatible/novel chemicals, including microbial insecticides, to mitigate the dilemma of environmental hazards and rise of resistance to pesticides.

The microbial insecticides can be equally as effective as synthetic chemicals to control insect pests. Sagheer et al., (2008) reported that integration of biocontrol agents and *Bacillus thuringiensis* (Bt) effectively reduced rice stem borer (*Scirpophaga incertulas*) populations. Similarly, the biopesticidal fungus *Metarhizium anisopliae* and bacteria *B. thuringiensis* (Bt) reduced populations of stem borers and leaf folders of rice in the laboratory and field (Shahid et al. 2003).

Nucleopolyhedrovirus have large, rod-shaped nucleocapsids with covalently closed, double- stranded DNA (Bilimoria, 1986; Federici, 1986). The nucleocapsid is surrounded by outer lipoprotein envelope, which can

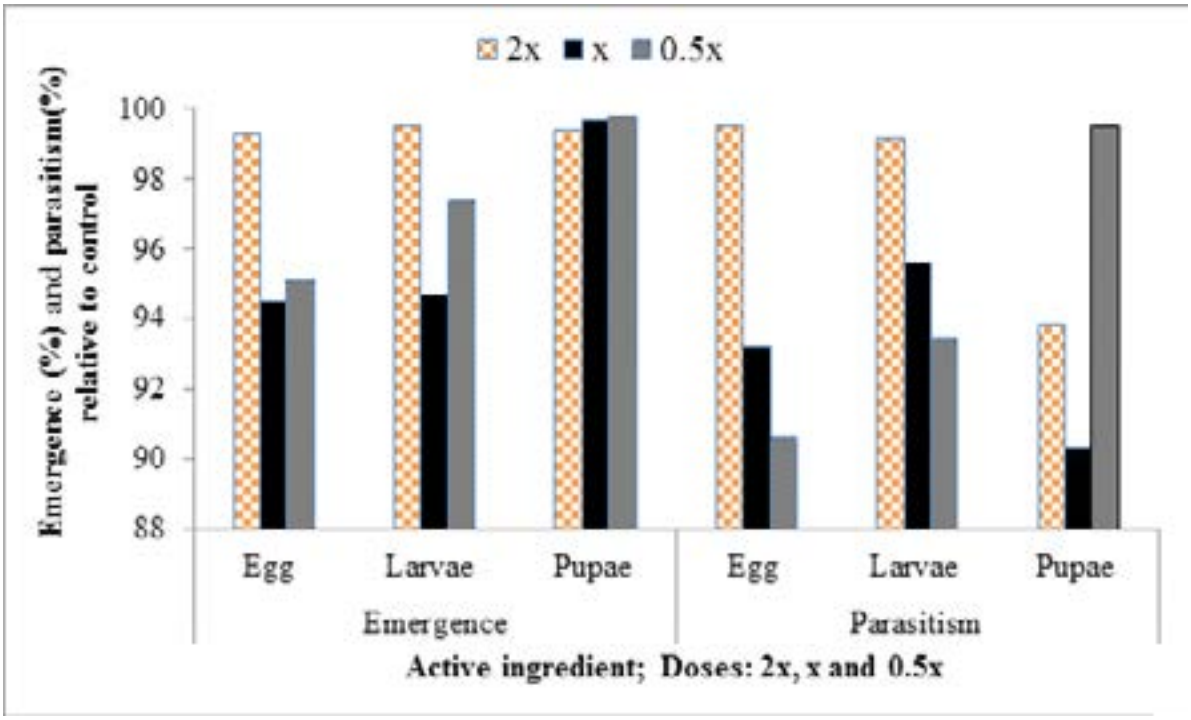

Figure 1. *Percent emergence (mean) relative to control of and percent parasitism (mean) relative to control by T. chilonis emerged from host eggs treated with HaNPV when parasitoid was at egg, larval and pupal stages*

Table 3. *Percent emergence (mean ± S.D) by T. chilonis in relation to stage and dose based on sqrt transformed data and mean comparison (Tukey HSD, p = 0.05 or 5%)*

	Mean ± standard deviation				
Stage	**Control**	**0.5x dose**	**x dose**	**2x dose**	**Mean**
Egg	88.00 ±4.22 ab	83.71 ±3.24 b	83.21 ±3.79 b	87.36 ±4.70 ab	85.57 ± 2.46 c
Larvae	92.09 ±2.86 a	89.70 ±3.37 a	87.23 ±3.43 ab	91.64 ±3.50 a	90.16 ± 2.21 a
Pupae	87.56 ±1.80 ab	87.38 ±1.23 ab	87.28 ±1.33 ab	87.06 ±4.39 ab	87.32 ± 0.21 b
Mean	89.22 ± 2.50 a	86.9 ±3.02 ab	85.91 ± 2.34 b	1.69 2.56 ab	-

Means sharing the same letter in each column/among columns are not significantly different from each other (Tukey's HSD, p > 0.05).

occur singly (single-embedded) or in groups (multiple-embedded) within the envelope. The virions are invisible by light microscope. Large occlusion bodies (OB), range from 1–15µm, comprised of a paracrys talline protein matrix formed by NPVs, are visible in a compound microscope, and occlude many virions protecting them to some degree during host-to-host transfer, although the OBs do not provide protection against sunlight (Benz, 1987; Ignoffo et al., 1989).

NPV_s are very successful to kill a variety of pest insects, although some insects survive and show only sublethal effects ranging from deformed pupae (Peng et al., 1997) to slower development, lower weight, reduced reproduction, and shorter life span (Rothman and Myers, 1996). There has been some research work carried out by various scientists assessing toxicity of microbial insecticides to beneficials, and most results of such work have demonstrated the compatibility of microbes with the other natural enemies.

Literature is not available on effects of NPVs on *Trichogramma chilonis*, although Sagheer et al., (2008) reported that application of bio-pesticides increased the effectiveness of *T. chilonis*. *Helicoverpa* nucleopolyhedrovirus or HaNPV is a very safe microbial insec-

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Table 4. *Percent reduction (mean ± S.D) in emergence over control of T. chilonis in relation to life stage and dose based on sqrt transformed data and comparison of means (Tukey HSD, p = 0.05 or 5%)*

Stage	Mean ± standard deviation			
	0.5x dose	x dose	2x dose	Mean reduction (%) ±S.E
Egg	4.78 ± 4.56 ab	5.32 ± 4.69 a	0.72 ± 2.21 bc	3.61 ± 2.51a
Larvae	2.55 ± 3.80 abc	5.20 ± 4.57 a	0.60 ± 0.45 abc	2.78 ± 2.31 a
Pupae	0.20 ± 0.18 c	0.32 ± 0.21 bc	0.57 ± 0.37 abc	0.36 ± 0.19 b
Mean	2.51 ± 2.29 ab	3.61 ± 2.85 a	0.63 ± 0.08 b	-

Means sharing the same letter in each column/among columns are not significantly different from each other (Tukey's HSD, p > 0.05).

Table 5. *Parasitism (mean ± S.D) by T. chilonis based on stage and dose and comparison of means (square root transformed data, Tukey HSD, p = 0.05 or 5%)*

Stage	Mean ± standard deviation				Mean parasitism
	Control	0.5x dose	x dose	2x dose	
Egg	23.17 ± 0.88 d	21.00 ±0.82 e	21.60 ±0.97 de	23.05±0.83 d	22.20 ±1.07 b
Larva	27.74 ±0.63 ab	25.92 ±0.88 bc	26.52 ± 0.74 ab	27.51±1.47 ab	26.92 ±0.85 a
Pupa	27.83 ±1.01 a	27.70 ±1.42 ab	25.14 ±2.08 c	26.12±1.45 abc	26.70 ±1.30 a
Mean	26.25 ±2.66 a	24.87 ±3.47 bc	24.42 ±2.54 c	25.56 ±2.17 ab	-

Means sharing the same letter in each column/among columns are not significantly different from each other (Tukey's HSD, p > 0.05).

Table 6. *Percent reduction (mean ± S.D) in parasitism relative to controls by T. chilonis and mean comparison (Tukey HSD, p = 0.05 or 5%)*

Stage	Mean ± standard deviation			
	0.5x dose	x dose	2x dose	Mean reduction (%) ±S.E
Egg	9.21±5.43 a	6.70±5.08 ab	0.57±1.26 c	5.49±4.44 a
Larvae	6.56±2.67 a	4.35±3.60 abc	0.97±0.84 bc	3.96 ±2.82 a
Pupae	0.48±0.46 c	9.52 ±6.93 a	6.06 ±5.37 ab	5.36 ±4.56 a
Mean	5.42 ±4.48 a	6.86±2.59 a	2.54 ±3.06 b	-

Means sharing the same letter in each column/among columns are not significantly different from each other (Tukey's HSD, P > 0.05).

ticide, as according to Moscardi (1999), viral insecticides present no threats to humans and are compatible with natural enemies of target pests, while at the same time HaNPV can effectively manage the target pests. For example, Ramteke and Gangurde, 2011 described that both fresh HaNPV (2x109 POBs/ml @

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250 ml/ha and 1x109 POBs/ml @ 500 ml/ha), and stored HaNPV formulations (stored for 1 year (2x109 POBs/ml @ 250 ml/ha and 1x109 POBs/ml @ 500 ml/ha) resulted in effectively reduced larval populations of *Helicoverpa armigera* and kept its population at minimum levels, leading to higher yields of pigeon pea.

The present research work demonstrated that HaNPV (microbial insecticide) is very compatible with both emergence and parasitism by *T. chilonis* adults emerging from treated eggs. Similarly, parasitism and emergence success of the *T. chilonis* were not influenced by the treatment of bacterium *Pseudomonas fluorescens* (Gandhi et al., 2005). Sagheer et al., (2008) reported that bioinsecticides (neem and Bt) can be integrated with *Trichogramma* egg parasitoids to enhance their bio-efficacy against rice leaf folder *Cnaphalocrocis medinalis*. Plant extracts and microbial formulations may effectively replace conventional synthetic insecticides.

Conclusion and Recommendations

HaNPV doesn't create any environmental or health hazards and can be stored under specific conditions for a long time. They are safe to people and wildlife, specificity is very narrow (Szewczyk, et al., 2006). The HaNPV is compatible with organic farming and can be used with Bt/chemical insecticide to suppress the target pests. This microbial product can be equally integrated with *T. chilonis* even at higher dose (2x). Therefore this microbial insecticide is widely recommended for integration with biological control to successfully control, *Helicoverpa armigera* (cotton bollworm) in cotton, lettuce, maize, tobacco and tomato.

Acknowledgements

The fellowship grant by the Higher Education Commission (HEC) of Government of Pakistan to the major author, Muhammad Ashraf Khan, PhD scholar, under the 5000 indigenous fellowship and also the laboratory research facilities provided to the scholar by the Nuclear Institute of Food and Agriculture (NIFA), Peshawar are gratefully acknowledged. This paper is part of PhD research work submitted as partial fulfilment for the requirement of degree of doctorate, PhD in Environmental Sciences.

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