Pak. J. Agri. Sci., Vol. 57(2), 439-445; 2020
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/19.8523
http://www.pakjas.com.pk

EFFICACY OF BIO-PESTICIDES AGAINST LARVAE OF *Trogoderma granarium* (EVERTS) (COLEOPTERA: DERMESTIDAE) ON MAIZE

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Kharap beetle, *Trogoderma granarium* (Everts) (Dermestidae: Coleoptera) is very notorious stored cereals insect pest, as well as of their commodities, throughout the world and can cause great economic losses. This research was planned to assess the efficacy of some bio-pesticides including entomopathogenic fungi (EPF) (*Metarhizium anisopliae* and *Isaria fumosorosea*), bacterial based insecticides (Spinetoram and Spinosad) and plant extract based commercial formulations (*Pongamia pinnata* and *Azadirachta indica*), alone and in combinations, against larvae of *T. granarium* under laboratory conditions. For mortality bioassays of bio-pesticides insect diet (maize kernels) was treated with three concentrations of each bio-pesticide i.e., for EPF (0.5×10^8, 1×10^8 and 1.5×10^8 conidia kg^{-1} grain) for bacterial based bio pesticide (0.5, 1.0 and 1.5ppm) and for plant extracts (50, 100 and 150ppm). Insect data regarding their percent mortality was recorded subsequently by exposure time of 7, 14 and 21 days in each case. Best result producing treatments of each bio-pesticide were identified and combine effect of best treatments of EPF and best treatments of remaining tested bio pesticides were evaluated for percentage mortality. Among all the tested bio-pesticides Spinetoram performed best against *T. granarium* larvae by giving 64.17% mortality with maximum concentration rate of 1.5ppm after 21 days followed by *M. anisopliae* whose mortal ranges 63% after 21 days with concentration rate of 2×10^8 conidiakg^{-1} grain remained closed to that given by Spinetoram. Spinosad proved as third best in efficacy while the results given by *A. indica* remained least significant. Combination of EPFs with Spinetoram, Spinosad, *A. indica* and *P. pinnata* against test insects showed significant difference as compared to their alone results. Higher mortality of targeted insect was observed in case of combine applications as compared to single application of each bio-pesticide. Highest mortality percentage of 79.33% was noted with the combination of *M. anisopliae* and Spinetoram after 21 days of exposure time. It is concluded that all the tested bio-insecticides are very effective to control *T. granarium*. Therefore, these bio-insecticides can efficiently be used in integrated pest management strategies against stored grain pests.

Keywords: Biopesticides, entomopathogenic fungi, bacteria, plant extracts, *T. gramarium.*

INTRODUCTION

Khapra beetle, {*Trogoderma granarium* (Everts) (Coleoptera: Dermestidae} is considered as one of most threatened insect pests around the world because of its rapacious feeding on almost all kind of the food stuffs (Sarmamy *et al*., 2011). The areas with low humidity and high temperature are more preferred by this insect pest (Ghanem and Shamma, 2007). The high economic significance of *T. granarium* can be well understand by considering that it has attained the prominence of quarantine pest so far (OEPP/EPPO, 1981) and is still under attention. The damage becomes intensifying when it is following up by attack of some secondary pests like *Aspergillus flavus* and *Ephestia cautella* (El-Nadi *et al.,* 2001). The larval stage of this insect is severe pest of oil seeds, cereals and to some extent pulses (El-Nadi *et al.,* 2001). The adult does not damage the food and only lay eggs while the larvae of *T. granarium* causes real damage (Musa *et al*., 2010). The losses caused by the attack of this beetle ranges 0.2– 2.9% during time duration of 1 month to 10.05 months (Irshad and Iqbal, 1994).

Conventionally synthetic fumigants and chemicals have been used mostly to keep safe stored grains and their products from the insect pests, but unfortunately extensive use of these measures has resulted in development of resistance in insects as well as problem of chemical residues in the products (Michalaki *et al*., 2006). Consequently, controlling insect by using traditional chemicals is become harmful ecologically and is expensive. Therefore, it has been created a developing quest for naturally ascending, globally safe and more economical means (Majumder *et al*., 2005). Biocides that are derived from plants or microorganisms have a significant prospective to be used as reduced risk insect pests control tactic (Regnault-Roger *et al*., 2002) as they are biodegradable, there action is selective, they are comparatively safer to non-target organism and finally have no negative impact on environment (Isman, 2001).

Many researchers have evaluated the toxic potential of microbial insecticides including EPF and bacterial based pesticides as well as of many plant extracts or their formulations against several stored grain insect pests but only few studies were done specifically against *T. granarium*. Just as Kumar *et al.* (2006) calculated EC_{50} value of 35.1µg $Insect^{-1}$ for methanolic extract of *P. pinnata* and Hasan *et al.* (2012) reported peak mortality of 14.36% with extract of *A. indica*, after 30 days post treatment (DPT) against *T. granarium*. In a latest research against *T. granarium*, Bilal *et al.* (2017) concluded that EPF have potential to control populations of *T. granarium* by reporting 66.80% larval mortality with application of *B. bassiana* at a dose rate of 9 x 10^7 $conidiakg^{-1}$ after 21 days post treatment (DPT).

Therefore, present study was planned to assess the toxicity of six biopesticides from three different sources i.e., Fungal, Bacterial and Plants against larvae of *T. granarium* to find out an effective and environmental friendly solution for this notorious pest.

MATERIALS AND METHODS

Insects: The test insect in given studies was *Trogoderma granarium* whose assorted samples were taken earlier from various storage structures of flour mills and grain market of Faisalabad for nurturing in the laboratory. A homogenous population was established at 60±70 % relative humidity and 30±2°C of temperature on purified food medium and third instar larvae were used in the whole study.

Formulations: Microbial insecticides including entomopathogenic fungi *Isaria fumosorosea* (1 x 10^8 $conidiag^{-1}$ (PAECILOMITE®), *Metarhizium anisopliae* 1 x 10^8 $conidiag^{-1}$ (PACER®), and botanical formulations including *Pongamia glabra* / *Pongamia pinnata* 2% EC / 20,000 ppm (DERISOM®), *Azadirachta indica* 1% EC/10,000 ppm (MARGOSOM®) were imported from Agri Life, Medak District. Hyderabad, India. Bacterial based insecticides, spinetoram {RADIANT® (spinosyn J and spinosyn L)} and spinosad {TRACER® (spinosyn A and spinosyn D)}, were purchased from Dow Agro Chemical company and Syngenta Pakistan Ltd., respectively.

Bioassays:

Toxicity of bio-pesticides against larvae of Trogoderma granarium using diet incorporation method: Sterilized maize, *Zea mays* L. was used as diet of *T. granarium* larvae. Three concentrations of each bio-pesticide i.e., for EPF (0.5 x10^8, 1 x 10^8 and 1.5 x 10^8 conidia kg^{-1} grain) for bacterial based insecticides (0.5, 1.0 and 1.5ppm) and for plant extracts (50, 100 and 150ppm) were applied on sterilized maize grains. There was a control treatment for each formulation. Treated commodity of 100 g was put into separate treatment jars. In total 40 test insects (3^{rd} instar larvae) were released into each treatment jar. The treated jars were placed in an incubator. Temperature and Relative Humidity was set at 30±2°C and 60-70%, respectively. The experiment was laid out in Completely Randomized Design (CRD) with three replications of each treatment. Percentage mortality data was collected after 7, 14 and 21 days post treatment.

Toxicity of biopesticides in combination against Trogoderma granarium larvae: Grains were treated with best concentrations regarding mortality of each entomopathogenic fungi. After drying, grains were put in the plastic jars and then best concentration of each remaining bio-pesticide, e.g. bacterial based and plant extract based formulations were mixed with treated grain separately. There was a control treatment for each formulation. Treated commodity of 100 g was put into separate treatment jars. In total 40 test insects (damaging stage) were released into each treatment jar. The treated jars were placed in an incubator. Temperature and Relative Humidity was set at 30±2°C and 60-70%, respectively. Completely Randomized Design (CRD) was used to conduct the experiment. Three replications were done in case of each treatment. Percentage mortality data was collected after 7, 14 and 21 days.

Statistical analysis: The data collected for toxicity and difference between experimental units as well as control treatments was analyzed using latest statistical software, R-Studio (R Development Core Team, 2005). For comparison of treatment means was performed using Tukey HSD test at a = 0.05.

RESULTS

Pathogenicity Bioassay: Mortality effect of six bio pesticides against larvae of *T. granarium* at different concentrations and exposure intervals of 7, 14 and 21 days (Table 1). In case of *M. anisopliae* maximum percent mean mortality (63%) was observed at 1.5×10^8 conidia concentration after 21 days followed by (52.17%) after 21 days at 1×10^8 conidia concentration while mean mortality percentage of (46.83%) was measured at 1.5×10^8 conidia concentration after 14 days. Minimum mean mortality percentage (8.17%) was noticed at lowest concentration of (0.5×10^8 conidia) after 7 days of exposure interval. Regarding *I. fumosorosea* maximum percent mean mortality (45.83%) was at 1.5×10^8 conidia concentration after 21 days followed by (36.67%) after 21 days at 1×10^8 conidia concentration while mean mortality percentage of (34.17%) was measured at 1.5×10^8 conidia concentration after 14 days. Minimum mean mortality percentage (7.5%) was noticed at lowest concentration of (0.5×10^8 conidia) after 7 days of exposure interval. For Spinetoram maximum mean mortality (64.17%) was attained at 1.5 ppm concentration after 21 days followed by (50.83%) mean mortality at 1 ppm concentration after 21 days. The mean mortality was (48.33%) at 1.5 ppm after 14 days and minimum mean

Table 1. Comparison of the mean values for percent mortality of entomopathogenic fungi, bacterial based bio pesticide and botanicals against larvae of *Trogoderma granarium* on maize after different exposure of time.

		Mean Mortality (%) ± SE			
	Conc.	7DAT	14DAT	21DAT	*F* value
M. Anisopliae	1	8.17±0.34e	25.83±0.43d	39.00±0.54c	
	2	13.33±0.34e	36.83±0.43c	52.17±0.43b	5.764**
	3	20.33±0.43d	46.83±0.49b	63.00±0.44a	
I. fumosorosea	1	7.50±0.89f	15.83±0.55e	25.00±0.48c	4.572*
	2	12.50±0.44ef	24.17±0.39cd	36.67±0.43b	
	3	17.50±0.58de	34.17±0.53b	45.83±0.28a	
Spinetoram	1	21.67±0.39e	29.17±0.49cde	35.00±0.39c	9.409**
	2	26.67±0.29de	34.17±0.34cd	50.83±0.92b	
	3	32.50±0.67cd	48.33±0.58b	64.17±0.43a	
Spinosad	1	19.17±0.39f	26.00±0.19de	39.17±0.49c	3.142*
	2	24.00±0.44ef	31.67±0.34d	47.50±0.48b	
	3	29.17±0.58de	42.17±0.20bc	56.67±0.31a	
A. indica	1	16.67±0.34f	23.50±0.19de	29.17±0.43c	0.445^{NS}
	2	20.00±0.35ef	28.33±0.29cd	34.17±0.39b	
	3	26.83±0.24cd	35.00±0.29ab	39.17±0.34a	
P. pinnata	1	21.67±0.53g	29.17±0.29ef	35.67±0.15bcd	0.037^{NS}
	2	25.00±0.48fg	33.17±0.20cde	39.50±0.19ab	
	3	30.67±0.63def	38.67±0.29abc	44.50±0.67a	

* = Significant, ** = Highly Significant, ^{NS} = Non-Significant

mortality percentage (21.67%) was noticed at lowest concentration of (0.5 ppm) after 7 days of exposure interval. The results of Spinosad showed maximum mean mortality of (56.67%) at 1.5 ppm concentration after 21 days followed by (47.5%) mean mortality at same concentration and exposure period. The mean mortality was (42.17%) at 1.5 ppm after 14 days and minimum mean mortality percentage (19.17%) was noticed at lowest concentration of (0.5 ppm) after 7 days of exposure interval. Results of *A. indica* were as, maximum percent mean mortality (39.17%) was at 150 ppm concentration after 21 days followed by (35%) after 14 days at 150 ppm concentration while mean mortality percentage of (34.17%) was measured at 100 ppm concentration after 21 days. Minimum mean mortality percentage (16.67%) was noticed at lowest concentration of (50 ppm) after 7 days of exposure interval. In case of *P. pinnata* maximum percent mean mortality (44.5%) was at 150 ppm concentration after 21 days followed by (39.5%) after 21 days at 100 ppm concentration while mean mortality percentage of (38.67%) was measured at 150 ppm concentration after 14 days. Minimum mean mortality percentage (21.67%) was noticed at lowest concentration of (50 ppm) after 7 days of exposure interval (Table 1).

***Comparative response of bio-pesticides against Trogoderma granarium larvae after different exposure of time*:** Noteworthy difference was observed among efficacy of all bio pesticides after each exposure period. After 7 days the maximum adult mortality (26.94%) was achieved with the application of Spinetoram followed by *P. pinnata*,

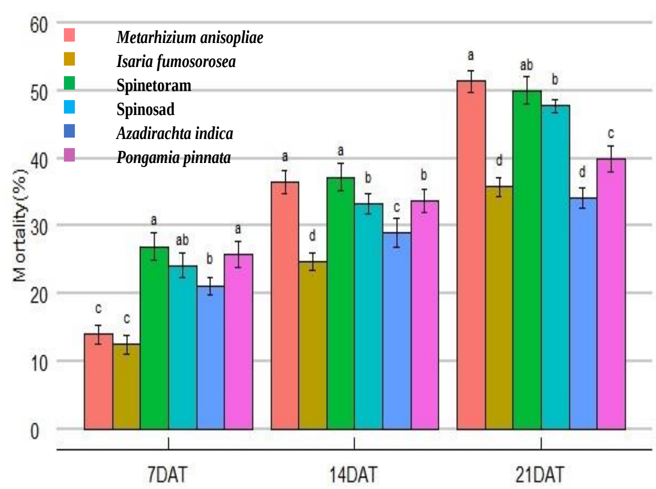

Figure 1. Pathogenicity of binary combination of fungus *M. anisopliae* with bacterial based bio pesticide and botanicals against *T. granarium*.

Spinosad and *A. indica* with 25.77, 24.11 and 21.16% mean mortality respectively. The lowest mortality of 12.5% was noticed in case of *I. fumosorosea* treatment followed by *M. anisopliae* (13.94% mean mortality). Spinetoram remained again most active after 14 days with resulting highest mortality of 37.22% while the least mortality percentage (24.72%) was noticed by application of *I. fumosorosea*. Mortality results of 21 days exposure period showed that maximum mortality (51.38%) was achieved with the application of *M. anisopliae*. Mean mortality of 50% was counted with Spinetoram followed by Spinosad (47.77%) and *P. pinnata* (39.88%) whereas *A. indica* remained least effective as compared to other tested bio pesticides and resulted in 34.17% mean mortality after 21 days of exposure intervals (Fig. 1).

The mean values of percent mortality by combinations of different concentrations after three exposure of time 7, 14 and 21 days. We concludes that the maximum percent mean mortality (79.33%) was obtained with combine application of best concentration of *M. anisopliae* and Spinetoram after 21 days followed by (72.5%) after 21 days with *M. anisopliae* and *P. pinnata* while mean mortality percentage of (71.17±0.38abc) was measured with *M. anisopliae* and Spinosad after 21 days. Minimum mean mortality percentage (33.33%) was observed with binary combination of best concentration of *M. anisopliae* and *A. indica* after 7 days of exposure period (Table 2).

Table 2. Mean mortality of *Trogoderma granarium* larvae on maize treated with best concentration of *Metarhizium anisopliae* in binary combination with bacterial based bio pesticide and botanicals against *T. granarium* after different exposure of time.

Exposure time (days) x Combinations	Mortality (%) ± SE
7 × (*M. anisopliae* + Spinetoram)	38.83±0.39f
7 × (*M. anisopliae* + Spinosad)	35.00±0.38f
7 × (*M. anisopliae* + *A. indica*)	33.33±0.34f
7 × (*M. anisopliae* + *P. pinnata*)	34.83±0.51f
14 × (*M. anisopliae* + Spinetoram)	62.50±0.51cd
14 × (*M. anisopliae* + Spinosad)	58.50±0.08de
14 × (*M. anisopliae* + *A. indica*)	50.67±0.80e
14 × (*M. anisopliae* + *P. pinnata*)	55.00±0.17de
21 × (*M. anisopliae* + Spinetoram)	79.33±0.72a
21 × (*M. anisopliae* + Spinosad)	71.17±0.38abc
21 × (*M. anisopliae* + *A. indica*)	69.33±0.71bc
21 × (*M. anisopliae* + *P. pinnata*)	72.50±0.66ab

F value calculated =1.056 ^{NS} (Non-Significant),

***Pathogenicity of binary combination of fungus I. fumosorosea with bacterial based bio pesticide and botanicals against Trogoderma granarium*:** The mean values of percent mortality by combinations of different concentrations after three exposure times 7, 14 and 21 days. We conclude that the maximum percent mean mortality (70%) was obtained with combine application of best concentration of *I. fumosorosea* and Spinetoram after 21 days followed by (67.5%) with *I. fumosorosea* and Spinosad while mean mortality percentage of (58.33%) was measured with *I. fumosorosea* and *P. pinnata* after 21 days. Minimum mean mortality percentage (32.33%) was observed with binary combination of best concentration of *I. fumosorosea* and Spinosad after 7 days of exposure period (Table 3).

Table 3. Mean mortality of *Trogoderma granarium* larvae on maize treated with best concentration of *Isaria fumosorosea* in binary combination with bacterial based bio pesticide and botanicals against *T. granarium* after different exposure of time.

Exposure Time (days) × Combinations	Mortality (%) ± SE
7 × (*I. fumosorosea* + Spinetoram)	34.17±0.53d
7 × (*I. fumosorosea* + Spinosad)	32.33±0.34d
7 × (*I. fumosorosea* + *A. indica*)	33.33±0.71d
7 × (*I. fumosorosea* + *P. pinnata*)	34.17±0.29d
14 × (*I. fumosorosea* + Spinetoram)	54.17±0.88bc
14 × (*I. fumosorosea* + Spinosad)	52.50±0.36bc
14 × (*I. fumosorosea* + *A. indica*)	46.67±0.34c
14 × (*I. fumosorosea* + *P. pinnata*)	50.83±0.24bc
21 × (*I. fumosorosea* + Spinetoram)	70.00±0.50a
21 × (*I. fumosorosea* + Spinosad)	67.50±0.38a
21 × (*I. fumosorosea* + *A. indica*)	54.17±0.46bc
21 × (*I. fumosorosea* + *P. pinnata*)	58.33±0.60b

F value calculated = 4.951** (Highly Significant)

DISCUSSION

The current study was carried out to evaluate the insecticidal effects of some bio-pesticides including entomopathogenic fungi (EPF), bacterial based insecticides and plant extract based commercial formulations, alone and in combinations, against larvae of *Trogoderma granarium*. The results noticed related to larval mortality of *T. granarium* were diverse. In case of single application of the treatments, maximum mean mortality (64.17%) was observed with the application of Spinetoram at a dose rate of 1.5ppm after 21 days. Vassilakos *et al*. (2015) studied toxic effect of spinetoram against adults of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Sitophilus granaries* and found *R. dominica* most susceptible with complete control at doze rate of 1 and 10ppm, after 14 days of application. The reason for less mortality of *T. granarium* larvae as compared to adults of *R. dominica* is seems to be that hairy body of larvae causes less contact with spinetoram. The second highest mortality percentage of 63% was noted by the application of *M. anisopliae* at dose rate of 2 x 10^8 conidia kg^{-1} grain after 21 days. A lot of published work is present regarding efficacy of EPF for the control of insect pests of stored grains (Batta, 2004a,b; Cherry *et al.*, 2005) but there is not any related reference about susceptibility of *T. granarium* to EPF to be linked with the results gained in our study except Khashaveh *et al*. (2011) who evaluated four Iranian isolates (IRAN-715C, IRAN-1018C, DEMI001, IRAN-437C) of *M. anisopliae* against *T. granarium* larvae and found all of them effective except IRAN-437C, while maximum results given by DEMI001 with lowest LC_{50} ($1x10^6$ conidia ml^{-1}). The mean mortality percentage of larvae and adults increased with the increase in conidial concentration. Our results for *T. granarium* show similarity to these findings. Likewise, Bello *et al*. (2001) reported 40% mortality by applying *M. anisopliae* against *S. oryzae* after 14 days of post-treatment with dose rate of that comes close to the mortality ranges gain in our research. Data analysis of Spinosad revealed mean mortality of 56.67% at maximum dose rate of 1.5ppm after exposure interval of 21 days that is significantly less as compared to the mortality achieved in case of Spinetoram and it was also reported by Vassilakos *et al.* (2012) that Spinetoram is more active than Spinosad for the control of *R. dominica* and got 100% mortality after 14 days of exposure interval even at very lower dose rates of Spinetoram. Application of *I. fumorososea* against larvae of *T. granarium* has given mean mortality of 45.83% after 21 days at maximum dose rate of 2×10^8 conidia kg^{-1} grain that is far below as compared to 92% adult mortality against *R. dominica* reported by Akmal*et al*. (2017) after 7 days but the difference is may be due to the higher dose rate of *I. fumosorosea* used in that study. Almost 45% larval mortality was achieved by single application of *P. pinnata* while among all tested bio-pesticides, the least larval mortality of 39.17% was attained by use of *A. indica* at maximum dose rate of 150ppm, after 21 days. Our results of *A. indica* shows resemblance to findings of Hassan *et al*. (2012) who has reported peak mortality of only 14.36% with extract of *A. indica*, after 30 days of exposure period against *T. granarium*. In another study, Ali *et al*. (2017) reported 24% mortality of *T. granarium* larvae against *A. indica* with a different dose rate, also the data was collected after 10 days. The findings of Kulkarni *et al.* (2015) are also in accordance with our results (*P. pinnata* is more effective than *A. indica* against *T. granarium* larvae), according to their report, ‘Among non-edible oils; Pongamia oil was observed to be superior over neem oil. Pongamia oil offered complete protection up to six months, Neem oil for four months. In this period there was 100 % mortality of the introduced larval population at dose rate of 1% (v/w).

For checking the toxicity of EPF in combination with other bio pesticides against test insects, best concentration of each fungus was applied along with best concentration of each bio pesticide. The Ccombinations of EPF with Spinetoram, Spinosad, *A. indica* and *P. pinnata* against test insects reveled significant differences as compared to their single response. Results revealed higher mortalities of test insect in case of their combinations as compared to when applied singly and highest mortality percentage of 79.33% was noted with the combination of *M. anisopliae* and Spinetoram after 21 days of exposure interval. The second-best result giving combination was of *M. anisopliae* and *P. pinnata*, with mean mortality of 72.5% after 21 days of exposure period followed by *M. anisopliae* and spinosad with observed mean mortality of 71.17% that is very close to combination of *M. anisopliae* and *P. pinnata*. The mean mortality noted with combine application of *M. anisopliae* and *A. indica* was 69.33%. Previously Musa *et al*. (2014) has checked the combined efficacy of *M. anisopliae* and *A. indica* against cucumber fruit flies with 81% mean mortality recorded and concluded that the effectiveness of these bio-pesticides could be increased by applying them in combination, as noticed in our study. Results of Mohan *et al.* (2007) also support our finding who reported synergistic effect of EPF and *A. indica* against *S. litura*. In case of combine application of *I. fumosorosea* and Spinetoram 70% mean mortality was resulted after 21 days of exposure period that is maximum among all the combinations of *I. fumosorosea* with other bio-pesticides. While the least performing combination was of *I. fumosorosea* and *A. indica* with mean mortality of 54.17% after 21 days. Our synergistic results regarding combine application of *I. fumosorsea* with other bio pesticdes shows similarity to the work of Riasat *et al.* (2013) which have found that *I. fumosorsea* shows additive effect when it was used in combination with DEBBM against *R. dominica*. Use of these bio pesticides in combination can be better option as compare to use them singly as all the combinations have shown synergistic effects.

***Conclusion*:** This in-vitro study determines the effectiveness of all the tested bio-pesticides for the control of *T. garanarium* larvae but bacterial based bio-pesticides (Spinetoram) can be more effective mean for efficient and quick control as it has shown highest control even after less exposure of time as compared to the other treatments. While as an efficient insect pest management (IPM) strategy against *T. granarium* combine utilization of Spinetoram and *M. anisopliae* as new biocontrol tool against agricultural insect pests, and the EPF have long effect because it can propagate themselves.

***Acknowledgment*:** We would like to thanks to Higher Education Commission Islamabad, Pakistan for their Financial Assistance under 5000 Ph. D. Indigenous Fellowship Program.

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[Received 10 Jan 2019: Accepted 30 April- 2019 Published 8 Feb.2020]