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Impacts of pesticides on Black kites, *Milvus migrans govinda* in Bundelkhand region of India

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ABSTRACT

Milvus migrans govinda is a helpful raptor that helps in controlling animals those are harmful for agriculture. It is a member of Accipitridae family. It's feeding habit involves small animal such as small mammals (mice and rats), snakes, lizards and insects. This shows that *Milvus migrans govinda* is a helpful raptor that helps in controlling animals that are harmful for agriculture. Organochlorine pesticides are well known as persistent contaminants that accumulate in the upper trophic levels of food chains. Pesticides injure birds both directly and indirectly and birds are often affected by a combination of different kinds of effects. When birds in the wild are harmed by pesticides, acute mortality, indirect impacts and sub-lethal effects occur together in a multi-faceted combination. Twelve *Milvus migrans govinda* found dead between 2007-2011 in Bundelkhand region were examined to investigate the health status, including the causes of death and the burden of organochlorine contaminants. Concentrations of organochlorine pesticides were examined by gas chromatography. Death of three *Milvus migrans govinda* attributed to contamination of pesticides found in lethal concentration due to bioaccumulation in food chain. Thus, lethal concentrations found in present study may be contribute a serious environmental factor affecting the survival of considered population. There is a possibility for some interesting research to come from this present research.

Keywords: Raptor, wild, pesticides, harmful, agriculture, mortality.

INTRODUCTION

Organochlorine pesticides have been used extensively in the control of insects from several years. Organochlorine pesticides are well known as persistent contaminants that accumulate in the upper trophic levels of food chains. Pesticides use to control grasshoppers and other insects, those are major part of their diet. Being top predators in the food chain, raptors are particularly susceptible to a variety of pesticides ingested by their prey accumulate in the raptors' bodies. Birds of prey are subject to the highest exposures of these chemicals which resulted in dramatic population declines of some species: in Europe and America, the effects of dichlorodiphenylotrichloroethane (DDT) on peregrine falcons (*Falco peregrinus*) have been well documented. These compounds have many and varied deleterious environmental effects (Edwards,

1975). Human initially overlooked the fact that the organochlorine pesticides are not specific poisons for insects and may kill other organisms. The use of pesticides in agriculture has often caused raptor mortality (Balcomb 1983; Henny and others 1987; Goldstein and others 1996; Mineau and others 1999). A close relationship between organochlorine insecticide residues in avian tissues and eggs and reduced reproductive success has been demonstrated for a variety of birds, both in controlled experiments and in field situations (Cooke, 1973). Warner *et al.*, (1966) reported that chemical pesticides cause serious sublethal effects during the reproductive stages of birds. Sublethal exposure may contribute to other causes of mortality such as trauma. The preying birds like Peregrine falcon, *Falco peregrinus*, Whooping crane, *Grus americana* and Bald eagle, *Haliaeetus leucocephalus* are subjected to secondary poisoning when they consumed prey. Pesticides and their residues can affect birds and their young directly or indirectly by contaminating food sources. Exposure to pesticides during reproductive stages affects hatching success and fledging survival, as well as increase the chance of reproductive failure.

Present study reveals the accumulation of persistent organochlorine pesticides in *Milvus migrans govinda* in Bundelkhand region. The chemical levels are compared with literature values in birds elsewhere. It should be encouraged raptors nesting, they will help to control populations of rodents and other pest species those are harmful for agriculture. Use of chemical pesticides should be avoided which can be harmful to raptors and other wildlife.

MATERIALS AND METHODS

STUDY AREA

Bundelkhand region is located between 23° 20' and 26° 20' N latitude and 78° 20' and 81° 40' E longitude bounded by the Yamuna in the north, the Chambal in the north west, erupted ranges of the Vindhya in the south east. Bundelkhand region including 13 districts of Uttar Pradesh and Madhya Pradesh. Bundelkhand region includes Jhansi, Lalitpur, Jalaun, Hamirpur, Banda and Mahoba in Uttar Pradesh and Sagar, Chhatarpur, Tikamgarh, Panna and Damoh in Madhya Pradesh including parts of Gwalior, Datia, Shivpuri and Chanderi (Figure 1).

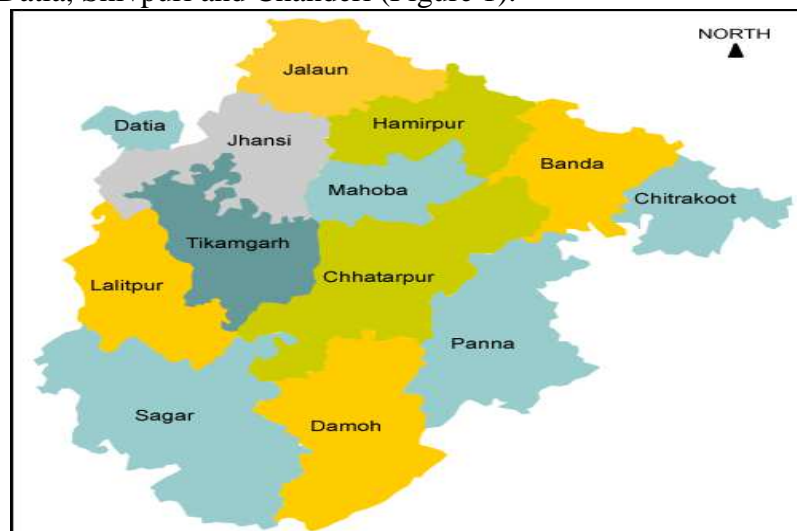


Figure 1: Map of study area “Bundelkhand region”

Source: <http://www.apnabundelkhand.com/>

Samples were homogenized with formic acid and n-hexane. Homogenized samples were dissolved in n-hexane and mixtures were shaken for an hour. Upper layers were separated and diluted with distilled water. Mixtures were passed through anhydrous sodium sulphate to remove moisture. Then extracts were rotary evaporated (bath temperature, 30 °C) to 1 ml and residue transferred

quantitatively with hexane into a 5 ml volumetric flask for the GC analysis. The recovery values were calculated from calibration graphs that were constructed from the concentration and peak area of the chromatograms obtained with standards of the organochlorine pesticides. Blank analysis were performed in order to check interference from the sample.

RESULTS AND DISCUSSION

Chronic low level organochlorines pesticides exposure affects the reproductive success of birds and changes their mating behaviour. The affected birds ignore territorial barriers, exhibit less attentiveness to young and decrease the extent of their home range (Fry, 1995). Twelve dead Black Kites, *Milvus migrans govinda* were found during study. Examination of stomach contents revealed that they had been gorging on grasshoppers and other insects. Liver and kidneys of all dead birds were analyzed for their levels of pesticides. The list of concentrations of pesticides residues in dead *Milvus migrans govinda* samples is given in Table 1.

Lethal concentrations of pesticides were not detected in MA1, MA2, MA6, MA7, MA8, MA9, MA10 and MA11. Three *Milvus migrans govinda* (MA3, MA4 and MA5) died obviously due to acute organochlorine pesticides poisoning.

Concentrations of pesticides in MAS were noted α -BHC 0.0024 ppm, heptachlor 1.6336 ppm, lindane 0.002878 ppm in liver and α -BHC 6.0490 ppm, heptachlor 0.0670 ppm, aldrin 4.94 ppm, β -HCH 1.16 ppm, endosulfane 1.618 ppm, dieldrin 9.82 ppm in kidney.

Concentrations of pesticides in MA3 were analyzed α -BHC 2.079 ppm, α -aldrin 204.48 ppm, heptachlor 2.60 ppm, γ -chlordane 2.24 ppm, dieldrin 69.39 ppm, endrin 6.53 ppm, DDD 4.52 ppm in liver and α -aldrin 66.8 ppm in kidney.

Concentrations of pesticides in MA4 were reported α -BHC 117.7 ppm, lindane 0.4889 ppm, heptachlor 4.00 ppm, dieldrin 60.96 ppm in liver and α -BHC 72.9 ppm, heptachlorepoide 0.0129 ppm in kidney.

Concentrations of pesticides in MA5 were noted α -BHC 77.6 ppm in liver and dieldrin 99.2 ppm, heptachlorepoide 2.74 ppm, endosulfane 0.0013 ppm. The resulting concentration of pesticides can be an important cause of death. Concentrations of pesticides in carcasses of *Milvus migrans govinda* were reported not significant when they compared with all districts and carcasses (Graph 1-2 and Table 2-3).

Table 1: Concentration of different pesticides in carcasses

Coding	Nature of sample	Pesticides	Concentration (ppm)
MA1	Liver, Kidney	ND	
MA2	Liver, Kidney	ND	
MA3	Liver	1. α -BHC 2. α -Aldrin 3. Heptachlor 4. γ -Chlordane 5. Dieldrin 6. Endrin 7. DDD	2.079 204.48* 2.60 2.24 69.39 6.53 4.52
	Kidney	1. α -Aldrin	66.8

MAS	Liver	1. α -BHC 2. Heptachlor 3. Lindane	0.0024 1.6336 0.002878
	Kidney	1. α -BHC 2. Heptachlor 3. Aldrin 4. β -HCH 5. Endosufan 6. Dieldrin	6.0490 0.0670 4.94 1.16 1.618 9.82
MA4	Liver	1. α -BHC 2. Lindane 3. Heptachlor 4. Dieldrin	117.7 0.4889 4.00 60.96
	Kidney	1. α -BHC 2. Heptachlorepoxyde	72.9 0.0129
MA5	Liver	1. α -BHC	77.6
	Kidney	1. Dieldrin 2. Heptachlorepoxyde 3. Endosufan	99.2 2.74 0.0013
MA6	Liver, Kidney	ND	
MA7	Liver, Kidney	ND	
MA8	Liver, Kidney	ND	
MA9	Liver, Kidney	ND	
MA10	Liver, Kidney	ND	
MA11	Liver, Kidney	ND	

*Higher concentration

Table 2: Variation in concentration of pesticides in carcass of *Milvus migrans govinda*

ANOVA					
Concentration					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	17944.116	11	1631.283	.701	.725
Within Groups	55823.152	24	2325.965		
Total	73767.268	35			

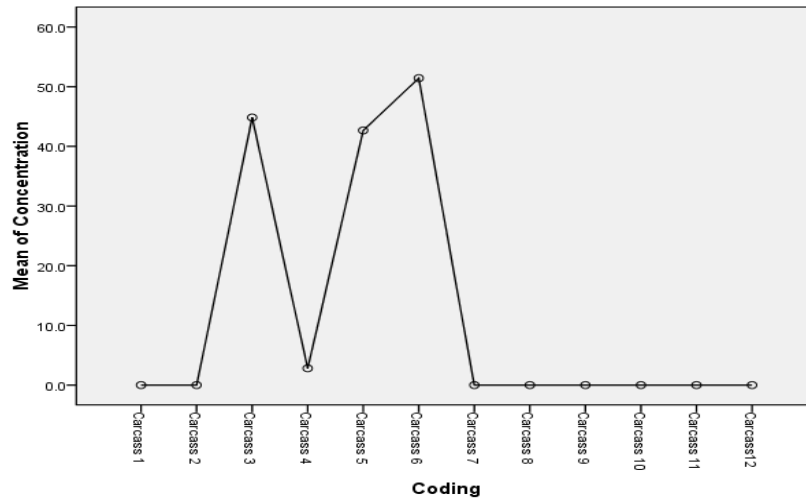
Oneway

Table 3: Variation in concentration of pesticides in liver and kidney collected from carcasses of *Milvus migrans govinda*

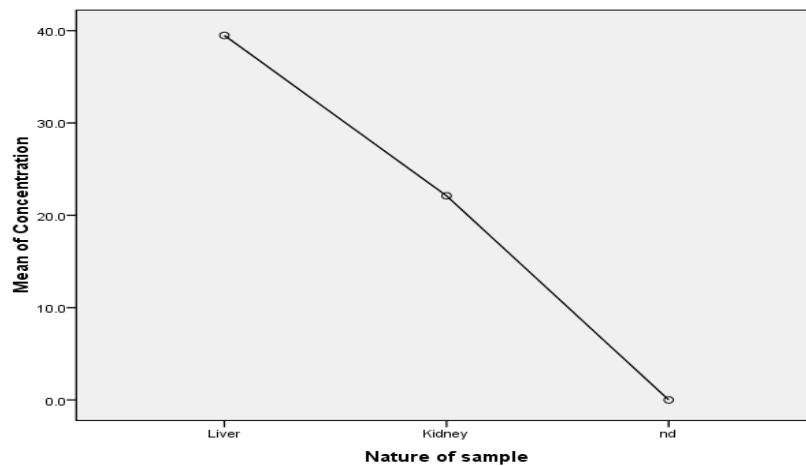
ANOVA					
Concentration					

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8459.044	2	4229.522	2.137	.134
Within Groups	65308.224	33	1979.037		
Total	73767.268	35			

Means plots



Graph 1: Mean of concentration of pesticides in carcass of *Milvus migrans govinda* in districts of Bundelkhand region



Graph 2: Mean of concentration of pesticides in liver and kidney from carcass of *Milvus migrans govinda*

Persistent organic pollutants such as organochlorine pesticides are distributed in food webs globally and due to their bioaccumulative properties and persistence, they biomagnify to their greater concentrations in higher-level consumers (Senthil Kumar *et al.*, 2002a). In several studies, raptors have been used to indicate local or regional contamination as I. D. Falkenberg *et al.*, (1994) investigated extent of organochlorine pesticide contamination in 3 species of predatory birds from the central regions of South Australia. Residues in both eggs and tissue, as well as measurements of shell thickness, were obtained for, Peregrine falcon, *Falco peregrinus*, Osprey, *Pandion haliaetus* and White-bellied sea eagle, *Haliaeetus leucogaster*. Organochlorine residues in some prey species were also measured. Residues of DDE and DDT in the predatory birds were found to be low to moderate. Dieldrin was present in most samples, but at low concentration. The degree of pesticide

contamination detected was considered sufficient to interfere with the reproduction of *F. peregrinus* in South Australia. James W. Parker *et al.*, (1976) investigated pesticide contamination and eggshell thickness of the mainly insectivorous Mississippi kite, *Ictinia mississippiensis* in Kansas, Oklahoma and Texas. Tissues contained small amounts of pesticides (DDT, DDE, dieldrin, aldrin) often used there in the past. Egg shell thinning has occurred and appears greatest in the south, where pesticide use probably has been greatest, but maximum thinning is minor and unlikely to affect reproductive success on a regional basis. Reichel *et al.*, (1984) analyzed 239 dead or moribund Bald eagles, *Haliaeetus leucocephalus* were collected from 32 states in the United States. They found that DDE concentrations were 3.3 $\mu\text{g/g}$ ww highest in a carcass and 1.3 $\mu\text{g/g}$ in brain. Concentrations of DDD (dichlorodiphenyldichloroethane), DDT, dieldrin, heptachlor epoxide, oxychlorane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, endrin, toxaphene, HCB, and mirex were $<1 \mu\text{g/g}$ and PCBs concentrations were highest in 5.3 $\mu\text{g/g}$ in carcass. Laura B. Rivera-Rodriguez and Ricardo Rodriguez-Estrella (2011) detected organochlorine pesticides residues in 28 Osprey, *Pandion haliaetus* nestlings from a dense population in Laguna San Ignacio, California. They found 16 organochlorine pesticides as hexachlorohexane, heptachlor, heptachlor epoxide, endosulfan, DDE, DDD, aldrin, dieldrin, endrin and others in lower concentration ranging from 0.000002 ppm-0.006856 ppm. They have been suggested that organochlorine pesticides also affects competitive interactions and population status over the long term in vertebrate species. Peakall (1996) reviewed the causes of death of Bald Eagles, *Haliaeetus leucocephalus* found dead in the U.S. by a network of federal, state, and private investigators from 1966 to 1983. The percentage of deaths attributed to dieldrin decreased following a ban on its use (i.e., 13% in 1966–70, 6.5% in 1971–74, 3.0% in 1975–77 and 1.7% in 1978–83). The levels of aldrin 16.8 ppm in Pheasants, *Phasianus colchicus* may be harmful has been demonstrated by USGS (1999). These hypotheses are supported by present study because pesticides are being excessively used by farmers without knowing their harmful effects. Despite the continuing usage of pesticides in Bundelkhand region, the exposure has not been studied previously. Bundelkhand region has experienced acute scarcity of water for agricultural and domestic use. Farmers rely primarily on subsistence rainfed crop agriculture and livestock production for their livelihood, with wheat, gram and oil seeds the predominant crops. Rabi crops area is about 69% as compared to 31% of kharif season because a large kharif area is kept as fallow. A tradition of *Anapratha* means letting loose animal for unrestricted grazing during kharif season which is against kharif cultivation in Bundelkhand region. The crops suffer from attack of various insect pests like grasshopper, termite, armyworm, wheat bug, wheat aphid etc. are common agricultural pests found in Bundelkhand region and represent a major threat to agricultural development. Thus, pesticides such α -BHC, α -aldrin, heptachlor, γ -chlordane, dieldrin, endrin, DDD, lindane, β HCH, endosulfan are used on a very large scale in Bundelkhand region. The feeding habits of *Milvus migrans govinda* foraging in agricultural lands and rubbish dumps therefore, exposure to organochlorine pollutants residues through feeding on contaminated is likely to be the major threat for this species. The present study was conducted to elucidate the accumulation pattern of α -BHC, α -aldrin, heptachlor, γ -chlordane, dieldrin, endrin, DDD, lindane, β HCH, endosulfan in *Milvus migrans govinda*.

Endrin is a nerve poison and dangerous for birds. It is used against grasshoppers and other insects. It is also used to control rodents and birds. It harms the nervous system. Residues of 0.8 ppm or more of endrin in brain mean death; 0.6 ppm or less mean survival; between was a zone of overlap. These criteria indicate that some wild birds of the US, particularly white pelicans in the northwest and two Bald eagles, *Haliaeetus leucocephalus* have been killed by endrin (W. H. Stickel *et al.*, 1979). It is rapidly assimilated by animals. Endrin penetrates the atmosphere by evaporation and can contaminate surface waters by drainage. Lethal concentration of endrin 6.53 ppm has been reported in liver of MA3 in present study. Heptachlor is an insecticide, used against insects like termites, grasshoppers, mosquitoes etc. Heptachlor is toxic and dangerous for wildlife, reported

lethal concentration in Mallard ducks, *Anas platyrhynchos* was 2080 ppm (R. H. Hudson *et al.*, 1984). Heptachlor and its more potent metabolite, heptachlor epoxide, have been found in the fat of fish and birds. They have also been found in the liver, brain, muscle and eggs of birds (World Health Organization, 1984). Heptachlor epoxide is stored mainly in fatty tissue, but also in liver, kidney and muscle tissues. Effects due to heptachlor exposure may include hyper excitation of the central nervous system, liver damage, lethargy, in coordination, tremors, convulsions, stomach cramps or pain and coma (A. G. Smith, 1991, Agency for toxic substances and disease registry, 1989). E. F. Hill *et al.*, (1986) reported lethal concentration of heptachlor 99 ppm in Japanese quail, *Coturnix japonica*. H. Kidd *et al.*, (1991) reported heptachlor were 450 ppm to 700 ppm in Bobwhite quail, *Colinus virginianus* and 250 ppm to 275 ppm in Pheasants, *Phasianus colchicus*. It was also reported to decrease the survivability of chicken eggs (US national library of medicine, 1995). In present study, heptachlor concentrations 2.60 ppm in liver of MA3, 1.6336 ppm in liver and 0.0670 ppm in kidneys of MA3, and 4.00 ppm in liver of MA4 were analyzed. Concentrations of heptachlor epoxide were reported 0.0129 ppm in kidney of MA4 and 2.74 ppm in kidney of MA5. Concentrations of heptachlor and heptachlor epoxide were reported below in lethal limit.

Endosulfan, a neurotoxic pesticide, is highly to moderately toxic to bird species. Administration of endosulfan by the dietary route resulted in lethargy, weakness and diarrhoea in Japanese quail, *Coturnix japonica* (Prakash *et al.*, 2009). The diarrhoea and the nervous symptoms produced by endosulfan are due to stimulation of the central nervous system (Hudson *et al.*, 1984). Endosulfan has been used in agriculture around the world to control insect pests including whiteflies, *Trialeurodes vaporariorum* aphids, *Acyrtosiphon pisum*, leafhoppers, *Eurymela distincta*, colorado potato beetles, *Leptinotarsa decemlineata*, and cabbage worms, *Pieris rapae*. Endosulfan is highly to moderately toxic to bird species, with reported values in Mallards, *Anas platyrhynchos* ranging from 31 ppm to 243 ppm (H. Kidd *et al.*, 1991 and R. H. Hudson *et al.*, 1984) and in Pheasants, *Phasianus colchicus* ranging from 80 ppm to greater than 320 ppm (R. H. Hudson *et al.*, 1984). Lethal concentration was reported 2906 ppm in Japanese quail, *Coturnix japonica* (E. F. Hill, 1986). In present study endosulfan concentrations were reported 1.618 ppm in kidney of MAS and 0.0013 ppm in kidney of MA5 which were reported below lethal limit.

Chlorodane is a pesticide applied to control termites and as a broad-spectrum insecticide on a range of agricultural crops, chlordane remains in the soil for a long time. Chlordane is highly toxic to birds. Lethal concentration for Bobwhite quail, *Colinus virginianus* is 83 ppm was reported. Lethal concentrations for chlorodane in Mallard ducks, *Anas platyrhynchos* 858 ppm and 331 ppm in Bobwhite quail, *Colinus virginianus* and 430 ppm in Pheasant, *Argusianus* were reported (R. T. Meister, 1992; US Environmental Protection Agency 1986; Lethal Diet Tox, 1975). Chlorodane concentration 2.24 ppm in liver of MA3 was reported which was found below lethal limit in this study.

Lindane is an organochlorine insecticide used widely as pre-harvest treatment on fruits, vegetables and other edible crops. Egg shell thinning and reduced egg production has occurred in birds exposed to lindane. It also harms the nervous system. Bhunya and Jena (1992) observed a significant increase in chromosome aberrations in chicks administered 100 mg/kg lindane. Lindane concentrations were reported 0.002878 ppm in liver of MAS and 0.4889 ppm in liver of MA4 which were reported below lethal limits.

HCH (Hexachlorohexene) is widely used to control a wide variety of pests of crops. The lethal dose for birds was estimated at 56 ppm (<http://www.inchem.org/documents/pims/chemical/pim257.htm>). HCH (Hexachlorohexene) concentration was reported 1.16 ppm in kidney of MAS which was reported below lethal limit.

BHC (Benzene hexachloride), a longer persistent, organochlorine pesticide is several times more lethal than DDT. The lethal dose is estimated at 50 ppm-100 ppm (<http://chemyq.com/En/xz/xz1/3439daekx.htm>). BHC was banned because of its longer persistence

and the accumulation of residues in food chain but it is still used in region. BHC concentrations 2.079 ppm in MA3, 117.7 ppm in liver and 72.9 ppm in kidney of MA4, 77.6 ppm in liver of MA5. Aldrin is a pesticide applied on soils against termites, locusts, grasshoppers, root eating worms and other pests. The acute toxicity of aldrin to avian species varies in the range of 6.6 ppm for Bobwhite quail, *Colinus virginianus* to 520 ppm for Mallard ducks, *Anas platyrhynchos*. Friend and Franson (2001) have collated mean sub and supra-threshold brain concentration data for aldrin/dieldrin and other organochlorine pesticides. Respective concentrations of <8 ppm or >18 ppm for dieldrin were documented however, these data contrast with experimental observations in Pheasants, *Phasianus colchicus*. Dieldrin concentrations in the brains of 50% (22 total) high-dose recipients that died within 48 hours of the start of the experiment ranged from 2.13 ppm to 4.25 ppm wet weight; equivalent values among survivors were 0.12 ppm –0.20 ppm. Vivian M. Mendenhall *et al.*, (1983) investigated that reproductive failure of raptors was related to organochlorine pesticides. They found dieldrin residues 5 ppm and 15 ppm in 2 raptor. They concluded that DDE was associated with egg shell significant thinning, egg breakage, embryo mortality and reduced production per pair and dieldrin contributed to their decline primarily through adult mortality. B. M. Mulhern *et al.*, (1970) reported pesticide residues in Bald eagles, *Haliaeetus leucocephalus* listed dieldrin as the likely cause of death of five Bald eagles, *Haliaeetus leucocephalus* in the Chesapeake Bay. The acute toxicity of dieldrin to avian species varies widely with the range of 26.6 mg/kg in pigeons to 381 mg/kg in mallard ducks (UNEP, 1995). In present study dieldrin reported from range normal 9.82 to lethal concentration 99.2 ppm. Death of three *Milvus migrans govinda* attributed to contamination of pesticides found in lethal concentration. Elevated contamination of these environmental contaminants suggested the existence of potential impacts on *Milvus migrans govinda* and wildlife. Thus, lethal concentrations found in present study may be contribute a serious environmental factor affecting the survival of considered population.

CONCLUSION

Milvus migrans govinda is a helpful raptor that helps in controlling animals that are harmful for agriculture. Present study attempted to establish the level of organochlorine pesticides in *Milvus migrans govinda* in Bundelkhand region. In present study, *Milvus migrans govinda* considered a good indicator of the effects of organochlorines pesticides on biological systems. This research investigated the levels of organochlorines pesticides in *Milvus migrans govinda* in Bundelkhand region.

The positive value of monitoring *Milvus migrans govinda* populations has been demonstrated with the observations of ecological injury that have occurred with misuse of pesticides. The negative effects of pesticides on *Milvus migrans govinda* have been signals for revision of laws and implementation of new regulations to prevent adverse effects.

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