ISSN 0971-930X
Journal of Biological Control
Published by Society for Biocontrol Advancement (Copyright 2000 SBA)

Journal of Biological Control, 26 (1): 43-48 , 2012

Research Article

Assessment of genotoxic potential of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* using *Acrida turrita* L. (Orthoptera: Acrididae) as a model

POOJA CHADHA*, SANDEEP K SANDHU, SHARANJIT KAUR, RASHID SALEEM and SANEHDEEP SAINI

Department of Zoology, Guru Nanak Dev University, Amritsar 143005, Punjab, India
Corresponding author E-mail: poojachadha77@yahoo.co.in

ABSTRACT: The adverse effect of fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the morphology of male meiotic chromosomes of grasshopper *Acrida turrita* was studied. Significant increase in the induction of aberrations as stickiness, clumping, pseudobridges, laggards, polyploid cells, extra element and stretching was manifested in treated grasshoppers as compared to control. Both the fungi induced a dose and time dependent increase in the number of chromosome aberrations. The frequency of production of percentage aberrations by *B. bassiana* in grasshopper was 6.50, 6.56, 7.24 with 10%, 7.12, 7.60, 8.16 with 20%, 9.45,10.04,10.25 with 30% after 6, 8, 10 hours respectively and 4.24, 5.60, 5.54 with 10%, 6.51, 6.98, 9.10 with 20% and 7.51, 8.21, 10.25 with 30% at 6, 8, 10 hours respectively by *M. anisopliae*.

KEY WORDS: *Beauveria bassiana, Metarhizium anisopliae, Acrida turrita,* Genotoxicity

(Article chronicle: Received: 7-9-2011 Revised: 30-1-2012 Accepted: 14-2-2012)

INTRODUCTION

Increasing awareness of the economic and environmental cost associated with the use of chemical pesticides has oriented the research towards biological control of different pests (Lomer *et al.,* 2001). An interest in entomopathogenic fungi as a potential substitute for chemical insecticides has greatly increased in agriculture (Ignoffo, 1975). Genera that have been most intensively investigated include *Entomopthora, Beauveria, Metarhizium, Verticillium* and *Lagenidium* (Srivastava, 2003). Among these, the green muscaridine fungus *Metarhizium anisopliae* is a common and widely distributed fungus. Its effectiveness has been tested against various insect pests as *Oryctes rhinoceros*, *Nilaparvatha lugens*, *Popillia japonica* and *Rhizotrogus majalis* (Sundara babu *et al.,* 1983; Aguda *et al.,* 1987; Krueger *et al.,* 1992). The white muscaridine fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *B. brongniartii* (Sacordo) Petch have been recorded to infect almost 500 host species belonging to Lepidoptera, Coleoptera, Hemiptera, Homoptera, Orthoptera and Diptera (Hall and Papierock, 1982; Moore and Prior, 1996). A lepidopteran derived isolate of *B. bassiana* proved to be pathogenic against green spider mites, *Mononychellus* spp. (Bartkowski *et al.,* 1988).

The parameters investigated till now in artificial bioassays are the effect of these microbial pesticides on the morphometric characters of insects. However, the sterlization effect of these entomopathogenic fungi has not been explored till now. *Acrida turrita* is most commonly distributed grasshopper in the area having 23 chromosomes which can be easily karyotyped and studied. Hence, the present study was carried out to explore the mutagenic effect of entomopathogenic fungi *B. bassiana* and *M. anisopliae* in spermatocytes of *A.turrita*.

MATERIALS AND METHODS

Insect Model Selected

Field collected *A. turrita* used as experimental model in the present study. Collection of grasshoppers was made in the month of July from the fields of Guru Nanak Dev University, Amritsar.

Entomopathogenic Fungus Selected

Two entomopathogenic fungi, *B. bassiana* (PDBC–Bb–5a) and *M. anisopliae* (PDBC–Ma–4) belonging to order Hypocreales and family Clavicipitaceae, were selected as a test material. The cultures of these fungi were procured from National Bureau of Agriculturally Important Insects, Bangalore and maintained on potato dextrose agar (PDA) medium. The PDA medium was prepared by mixing

3.9 g of PDA in 100 ml. of distilled water in a conical flask. After solidification within one hour PDA slants were kept in BOD for 24 hours at 28–30°C. The medium was inoculated with fungal spores and the slants were kept undisturbed for 10 days in BOD at 28–30°C. The fungal suspension was prepared by adding the mixture of one drop of Tween 80 in 10 ml of distilled water to fungal slants. The suspension was filtered through double layered muslin cloth. Spores of the fungal suspension were counted in the Neubaur's chamber of Haemocytometer. Stock solutions of *B. bassiana* and *M. anisopliae* were prepared from the above preparation at 7.9×10^7 spores/ml and 8.7×10^7spores/ml, respectively. Stock solution was diluted with distilled water to prepare 10% ($7.9x10^6$Spores /ml.), 20% ($1.58x10^7$ spores /ml) and 30% ($2.37x\ 10^7$ spores /ml) solutions.

Treatment of grasshoppers with fungal suspension

Adult grasshoppers were injected with 1ml of different concentrations of fungal suspension i.e. 10%, 20% and 30%

Table 1. Different type of chromosomal aberrations induced by *Metarhizium anisopliae* in *Acrida turrita* with different doses and at different time intervals

Time in hours	Conc.	No. of cells	Types of aberrations							Total ab.	Percentage ab.
			Clum.	Psb.	E. Ele	Sti.	Lag.	Gaps	Po.p.		
6	control	732	6	0	0	3	0	0	0	9	1.24
	10%	771	9	6	0	9	3	3	3	33	4.24*
	20%	672	9	3	0	18	3	3	9	45	6.51*
	30%	759	6	9	0	21	9	6	6	57	7.51*
8	control	936	6	0	0	6	0	0	0	12	1.23
	10%	753	12	3	6	9	3	6	3	42	5.60*
	20%	765	12	6	3	15	6	6	6	54	6.98*
	30%	783	6	12	3	18	0	12	12	63	8.21*
10	control	810	3	3	0	3	0	0	0	9	1.11
	10%	807	12	6	0	6	3	6	12	45	5.54*
	20%	780	12	9	3	12	9	12	15	72	9.10*
	30%	744	12	6	6	30	9	6	9	78	10.2*

*Significantly higher than control

Clum.–clumping, Psb.– pseudobridges, E.Ele– extra element, Sti.– stickiness, Lag.–laggards, po.p– polyploidy, ab.–aberations, conc–concentration

Table 2. Different types of chromosomal aberrations induced by *Beauveria bassiana* in *Acrida turrita* with different doses and at different time interval

Time in hours	Conc.	No. of cells	Types of aberrations						Total ab.	Percentage ab.
			Stic.	PsB.	Lagg.	Gaps.	Clum.	Stret.		
6	control	923	7	0	0	1	3	0	11	1.19
	10%	908	28	0	2	8	16	5	59	6.49*
	20%	927	39	2	4	3	15	3	66	7.11*
	30%	898	47	0	2	6	19	11	85	9.46*
8	control	906	7	1	0	0	1	0	9	0.99
	10%	883	30	2	3	8	7	8	58	6.56*
	20%	893	37	1	1	3	18	8	68	7.61*
	30%	867	49	0	1	7	17	13	87	10.03*
10	control	918	10	0	0	0	1	1	12	1.30
	10%	869	38	2	2	7	13	1	63	7.24*
	20%	919	45	1	4	4	12	9	75	8.16*
	30%	909	47	0	1	9	20	17	93	10.23*

*Significantly higher than control

with the help of insulin needle. They were dissected after the interval of 6, 8 and 10 hours. Tween 80 was taken as control material and injected in similar way as fungal suspension.

Chromosome Preparation

Air drying technique followed by Yadav and Yadav (1990) was used with minor modifications fro chromosomal studies. Testes were dissected out from the grasshopper and kept for 8-10 minutes in a saline solution containing 4–5 drops of colchicine. Hypotonic treatment with 1% sodium citrate was done for 20 minutes and then follicles were kept in fixative (3 methanol: 1 glacial acetic acid) for 30 minutes. Follicles were squashed in 45% acetic acid, spread on a slide, dried and stained in Giemsa stain for 20 minutes. Slides were washed with distilled water, dried and observed under microscope. For each concentration of fungal suspension as well as for each time interval experiment was repeated three times.

Screening of Slides

After temporary preparation, slides were examined under CX31 microscope at 100x and photographed.

Statistical Analysis Applied

Data was analyzed through student's t-test.

RESULTS AND DISCUSSION

We intended to investigate the dose and time response relationships between the fungi and chromosomal changes. Approximately 700–900 cells were observed in each category and various kinds of chromosomal anomalies were observed. The results obtained from the present investigation of structural chromosome aberrations induced by *B. bassiana* and *M. anisopliae* in *A. turrita* testicular cells are presented in tables 1–3.

Different kinds of chromosomal aberrations induced by *B. bassiana* and *M. anisopliae* in *A. turrita* with different doses at different time intervals respectively. The types of aberrations induced were found to be clumping Clum.-clumping, Psb.- pseudobridges, Sti.- stickiness, Lag.-laggards, stret.- stretching, ab.-aberations, conc-concentration pseudobridges, gaps, stretching, laggards, polyploidy, extra element and stickiness (fig.1). The stickiness between the chromosomes was found to be most common type of aberration. On the other hand occurrence of extra element and polyploid cell was observed only in *M. anisopliae* treated germ cell of *A. turrita.* The number of aberrations was found to be increased with increase in concentration as well as with time interval.

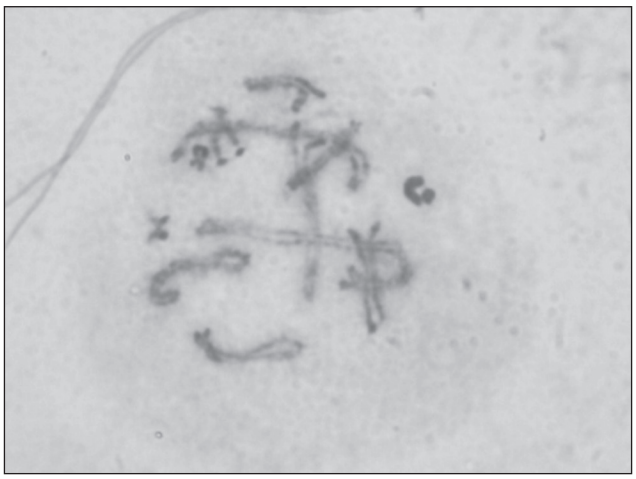
Table 3 reveals mean percentage values of chromosomal aberrations induced by different concentrations of *B. bassiana* and *M. anisopliae* in *A. turrita* varied at different time intervals. The mean values of chromosomal aberrations were found to be significantly higher ($p<0.01$) among all the treated concentrations at different time intervals as compared to control (student's' test). Two way analysis of variance (ANOVA) was applied and two way interaction (concentration x time) was also calculated. F ratios were computed for both fungus treatment (F being 22.7317 for *M. anisopliae* and 571.0292 for *B. bassiana*) and the interaction was found to be significant ($p<0.01$) in both cases (fig. 1 and 2).

In earlier studies on genetic toxicology, aflatoxin (metabolite of the fungus *Aspergillus flavus*) was found to induce similar kind of chromosomal aberrations in grasshopper *Oxya velox* and *ceracris deflorata* (Yadav and Yadav, 1984). Ohnuki (1956) reported chromosomal aberrations like stickiness and coalescence in grasshopper under the influence of the sarkomycin (an antibiotic produced from microorganisms in the soil). It was found to induce the chromosomal damage at metaphase. Cyclophosphamide and *Bacillus thuringiensis* were also found to induce chromosomal aberrations like chromatid breaks, gaps and deletions in germ cells of grasshopper, *Oxya chinensis*. Mohanty *et al.,* (2007) reported chromosomal aberrations like breaks, stretching, woolly appearance, stickiness, clumping and laggards in grasshopper, *Poecilocerus pictus* (au) treated with pesticide chlorpyriphos. Similar kinds of chromosomal aberrations were reported in the neuroblast of the grasshopper embryo, *Chortophaga viridifasciata* after the treatment with mitomycin C (Ferguson *et al.,* 2006).

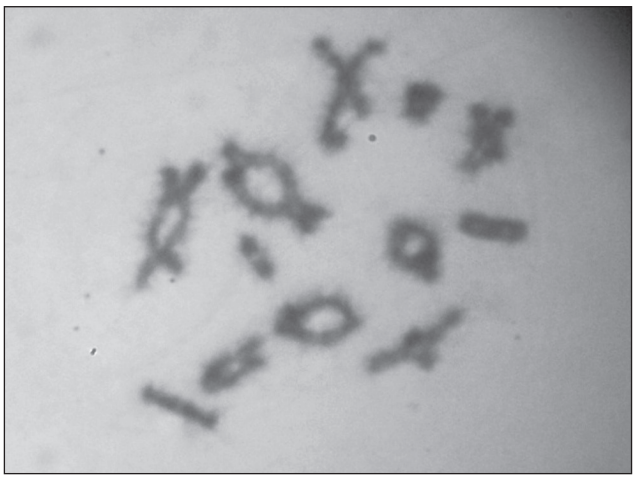
Stickiness observed in present study is a physiological phenomenon usually affected by many chemicals and physical agents in meiosis. According to Darlington and La Cour (1942) chromosome stickiness is attributed to excessive nucleic acid charge and failure of end gene reproduction. But, Saha and Khudabaksh (1974) attributed stickiness to the disturbance of DNA metabolism of cell or the oxidation reduction system of cell nucleus. It may result in fragmentation of chromosomes from the stress of anaphase movement or in bridge formation when fail to separate. Polyploid cells originated as a consequence of either spindle failure or failure of cytokinesis or both. The laggards are formed due to terminal stickiness of chromosomes which causes irregularities in timely separation of homologues to the poles. Pseudobridges observed might be formed due to the failure of chiasmata in a bivalent to terminalize and so chromosomes get stretched between the poles (Saylor and Smith, 1966). The presence of a bridge with or without fragments in both anaphaseI and anaphase2 could be because of paracentric inversions or may be due to fusion of sticky chromosomes (Sinha and Godward, 1972).

As the results of present study clearly suggested that *B. bassiana* and *M. anisopliae* are quite effective in inducing chromosomal abnormalities. The induction of different chromosomal anomalies by both the fungi may be due to the different toxins released by fungi. As the mode

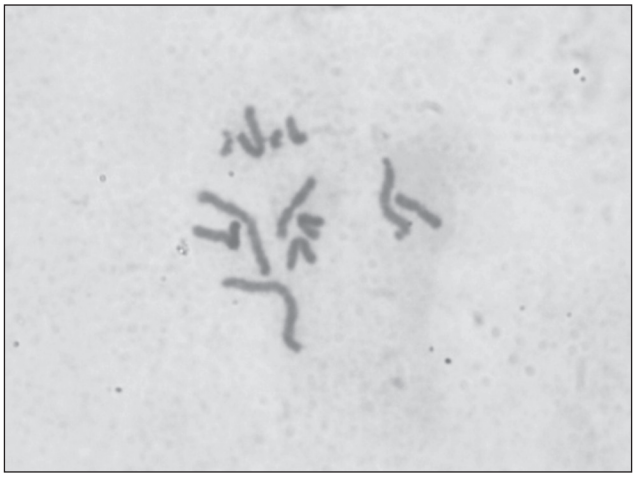
of colonization and growth of both the fungi in insects was reported to be yeast like the growth was very fast in insect body and released toxins in very short time (Roy *et al.,* 2005). *B. bassiana* was reported to release toxins as beauvericin, beauveriolides, bassianolide, isarolides and pigments like tenellin and bassianin and oxalic acid in culture media (Roberts, 1981). These toxins were reported to have antimicrobial, insecticidal, cytotoxic and apoptotic activity in different in vitro cultures (Klaric and Pepeljnjak, 2005 and Klaric *et al.,* 2008). *M. anisopliae* is also reported to secrete toxins viz. destruxins A, B, C, D and E and desmethyldestruxin B (Suzuki *et al.,* 1966).



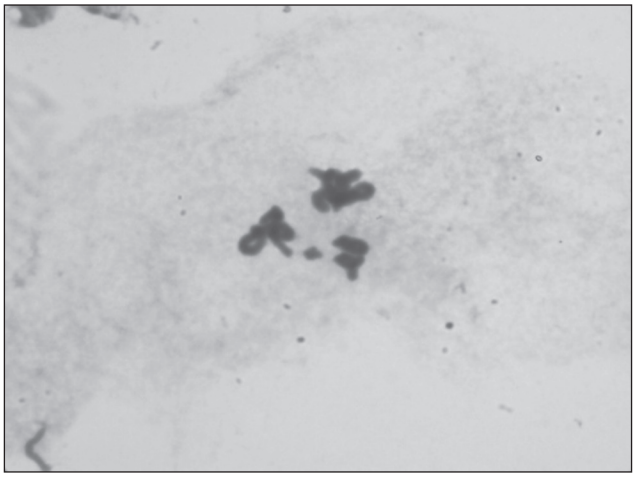
a



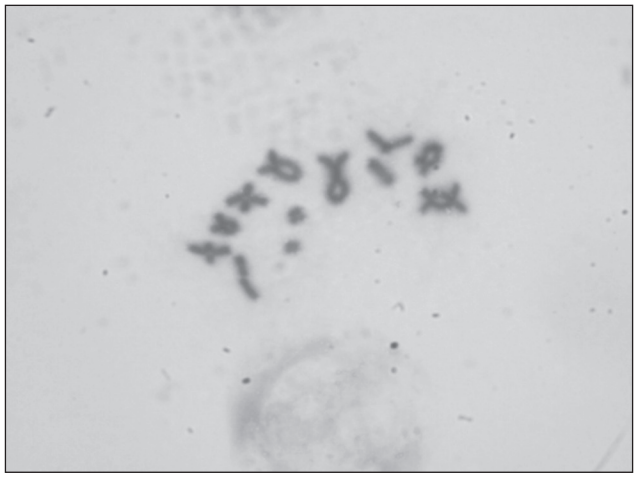
b



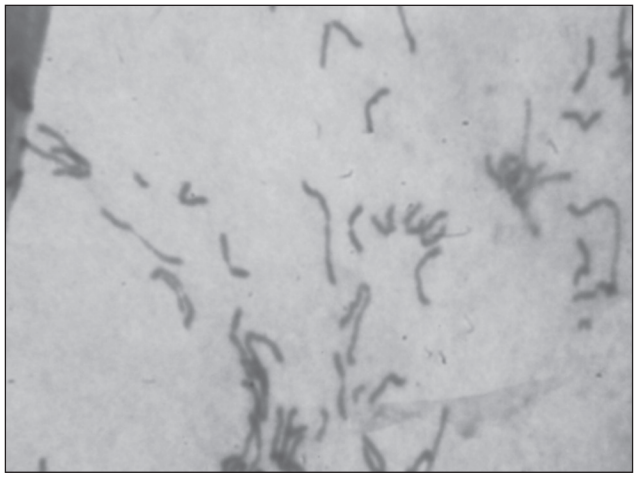
c



d



e



f

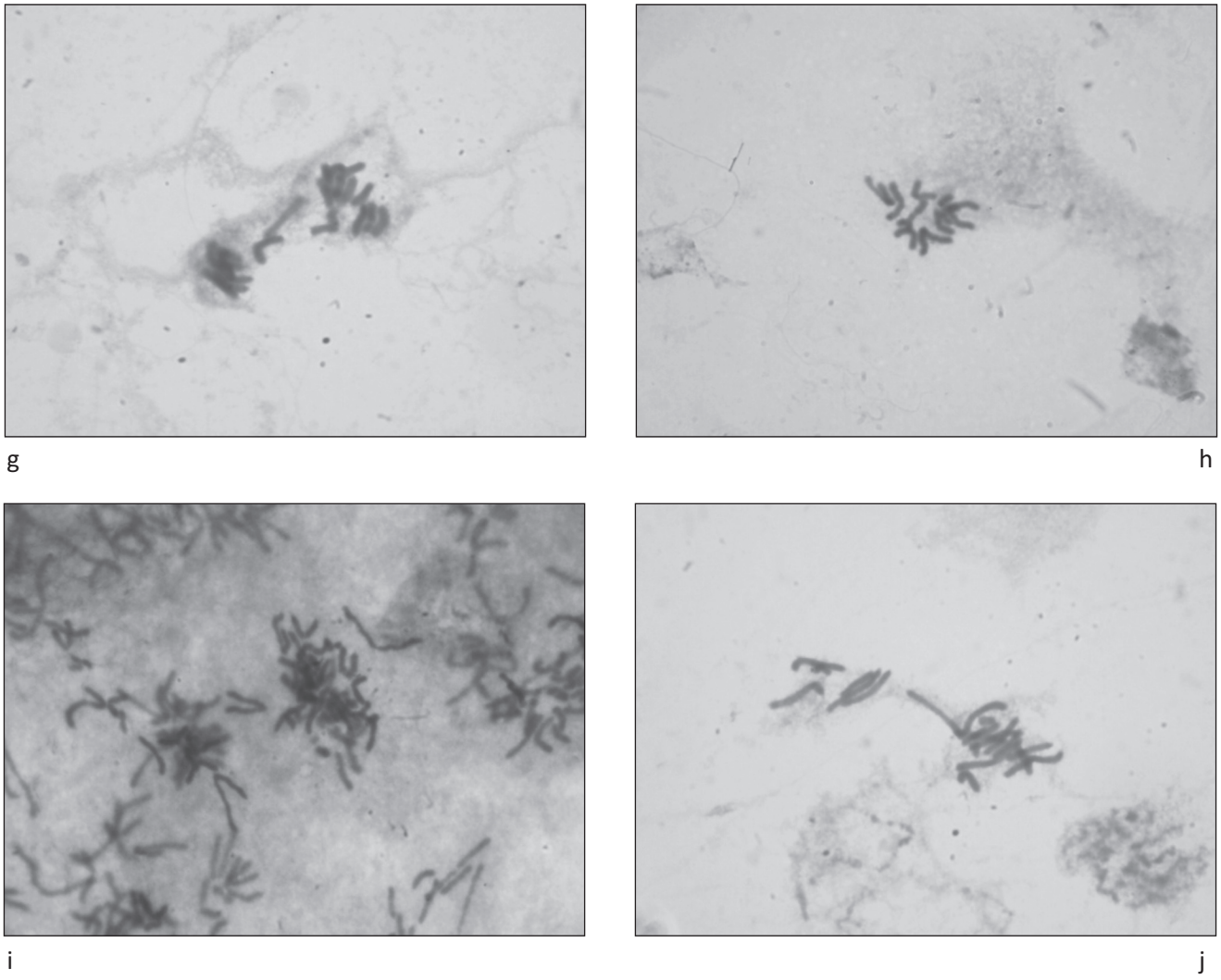


Fig. 1. Microscopic analysis of genetoxic potential of Beaveria bassiana

(a–c) Chromosomes in control group at different meiotic stages
(d) Clumping at diplotene stage
(e) Showing extra element
(f) Chromosome gaps
(g) Laggards at anaphase
(h) Pseudobridge at anaphase
(i) Stickiness and polyploidy
(j) Stretching of chromosomes at metaphase

ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Zoology, G.N.D.U. Amritsar for providing laboratory facilities and to Punjab State Council for Science and Technology for providing funds to carry out this project.

REFERENCES

Aguda RM, Rombach MC, Im DJ, Shepard BM. 1987. Suppression of populations of the brown planthopper, *Nilaparavatha lugens* (Stal.) (Hom: Delphacidae) in field cages by entomopathogenous fungi (Deuteromycotina) on rice in Korea. *J Appl Entomol.* **104:** 167–172.

Bartkowski J, Odindo MO, Otiens WA. 1988. Some fungal pathogens of the cassava green spider mites *Mononychellus* spp. (Tetranychidae) in Kenya. *Insect Sci Applic.* **9:** 457–459.

Darlington CD, La Cour LF. 1942. *The handling of chromosomes*. Allen & Unwin, London.

Ferguson MJ, Gaulden ME, Seibert GB. 2006. Chromosome fragments and other abnormalities induced by mitomycin C in the neuroblast of *Chortophaga viridifasciata*. *Environ Mutagen.* **7:** 547–561.

Hall RA, Papierock B. 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. *Parasitology.* **84:** 205–240.

Ignoffo, CM, 1975. Entomopathogens as insecticides. *Environ Letters.* **8:** 23–40.

Klaric MS, Pepeljnjak S. 2005. Beauvericin: chemical and biological aspects and occurrence. *Arch Ind Hyg Toxicol.* **56:** 343–350.

Klaric MS, Rumora L, Ljubanovic D, Pepljnjak S. 2008. Cytotoxicity and apoptosis induced by fumonisin B (1), beauvericin and ochratoxin A in porcine kidney PK 15 cells: effects of individual and combined treatment. *Arch Toxicol.* **82:** 247–255.

Krueger SR, Villani MG, Martins AS, Riberts DW. 1992. Efficacy of soil applications of *Metarhizium anisopliae* (Metsch.) Sorokin conidia and standard and lyophilized mycelial particles against scarab grubs. *J Invertebr Pathol.* **59:** 54–60.

Lomer CJ, Bateman RP, Johnson DL, Langewald J, Thomas M. 2001. Biological control of locust and grasshopper. *Annu Rev Entomol.* **46:** 667–702.

Mohanty PK, Bhanja M, Raj K, Otta SN. 2007. Genotoxic effect of chlorpyriphos on germ cells of short horned grasshoppers *Poecilocerus pictus* F. *Cytology* **72:** 397–400.

Moore D, Prior C. 1996. Mycoinsecticides. In: *IPM system in agriculture*, vol. **2**: Biocontrol in Emerging technology. (Eds.) RK. Upadhayay, KG. Mukerjii and RL. Rajak. Aditya Books Private Ltd., New Delhi, pp. 25–56.

Ohnuki Y, 1956. A phase microscopy study on the effect of sarkomycin upon dividing spermatocytes of grasshoppers, *Japan J Genet.* **31:** 197–200.

Roberts DW. 1981. Toxins of entomopathgenic fungi. In: *H.D. Burges (Ed.), Microbial Control of Pests and Plant Diseases (1970-1980)*. Academic Press, London, U.K., pp. 441–446.

Roy HE, Vega F E, Chendler D, Goettel MS, Pell JK. 2005. Ecology of fungal entomopathogens. Springer Dordrenht Heidelberg London. Newyork.

Saha AK, Khudabaksh AR. 1974. Action of 2,4' – dihydro-xychalcone on grasshopper spermatocytic chromosomes. *Cytology* **39:** 357–366.

Saylor LG, Smith BN. 1966. Meiotic irregularities in species of interspecific hybrids in *Pisum. American J Bot.* **53:** 453–468.

Sinha SSN, Godward MBE. 1972. Radiation studies in *Lens culinalris, Indian J Genet.* **32:** 331–339.

Srivastava RP. 2003. Status of adoption of biopesticides and biocontrol agents in Indian agriculture (V. Singhal). In: *Biopesticides and bioagents in Integrated Pest Management of Agricultural crops*, International Book Distribution Co. (Publishing division) pp. 1.

Sundara Babu PC, Balasubramanian M, Jayaraj S. 1983. Studies on the pathogenicity of the *Metarhizium anisopliae* (Metschnikoff) Sorokin var. *major* Tulloch on *Oryctes rhinoceros* (L.) *TNAU Res Publ. I.*

Suzuki A, Kuyama S, Kodair Y, Tamura S. 1966. Structural elucidation of destruxins A. *Agric Biol Chem. (Tokyo)* **30:** 517–518.

Yadav JS, Yadav, AS. 1984. Aflatoxin induced structural aberrations in grasshopper chromosomes, *Environment and Biotic Interaction* (Proc of 1V All India Symposium of Environmental Biologists, Dec. 1984) A.K. Dutta Gupta & R.P. Maleyyar (Eds.), 52–57.

Yadav, JS, Yadav AS. 1990. X-autosome fusion in catantopine grasshoppers (Acridoidea: Orthoptera). *Cytobios* **61**: 21–30.