

Key Indicator- 3.3 Research Publications and Awards (25)

3.3.2 Number of papers published per teacher in the Journals notified on UGC website during the last academic year (10)

S.No	S.No. (in data template)	Title of paper	Name of the author/s	Department of the teacher	Name of journal	Year of publication	ISSN number	Link to website of the Journal
1	20	Adhure vyaktiyon ki poorn abhivryakti: Aadhe adhure	Dr.Sunita	Hindi	Shodh Disha	October-December 2021	0975-735X	http://kavitakosh.org/kk/%E0%A4%B6%E0%A5%8B%E0%A4%A7%E0%A4%A6%E0%A4%BF%E0%A4%86%E0%A4%BE_%E0%A4%AE0%A4%ED%A5%8D%E0%A4%B0%E0%A4%BF%E0%A4%95%E0%A4%BE
2	52	Siddharth Gautam Ki Gautam Se Bodhisattva Banne Ki Yatra	Dr. Poonam	History	Shodh Disha	2022	0975-735X	http://kavitakosh.org/kk/%E0%A4%B6%E0%A5%8B%E0%A4%A7%E0%A4%A6%E0%A4%BF%E0%A4%86%E0%A4%BE_%E0%A4%AE0%A4%ED%A5%8D%E0%A4%B0%E0%A4%BF%E0%A4%95%E0%A4%BE
3	71	Immunostimulatory Effect of Ascorbic acid, Alpha Tocopherol and their combination on Asian Catfish, <i>Clarias batrachus</i> (LINN. 1758)	Ankita Singh and Seema Jain	Zoology	Journal of Experimental zoology, India	2022	0972-0030	https://connectjournals.com/
4	72	Immunomodulatory Effect of Vitamin C and E on Non-Specific Immune Parameters in fishes ; A Review.	Ankita Singh and Seema Jain	Zoology	South Asian Journal of Experimental Biology	2022	2230-9799	http://www.sasjeb.com/
5	73	Therapeutic Role of <i>Spirulina platensis</i> against Cadmium sulphate Toxicity in the Histopathology of Liver of <i>Clarias Batrachus</i> (LINN.)	Garima Pundir and Pragati	Zoology	Journal of Experimental zoology India	2022	0972-0030	https://connectjournals.com/
6	74	Protective Effect of <i>Spirulina platensis</i> in the Histopathological Alteration in the kidney of <i>Clarias batrachus</i> against Cadmium Sulphate toxicity.	Pragati and Garima Pundir	Zoology	Journal of Experimental zoology India	2022	0972-0030	https://connectjournals.com/jez
7	76	Efficacy of some botanical powders against rice weevil <i>Sitophilus oryzae</i> infesting sorghumseed under storage conditions.	Shashi Bala and Rashmi Mishra	Zoology	Journal of Entomological Research	2022	0974-4576	https://www.researchjournals.com/impact/details/21100210912DOI:105958/0974-4576.2022
8	77	Effect of Castration on photo periodic Bunting (<i>Emberiza bruniceps</i>) induced Food Intake and Fattening in Redly	Kalpna Chaudhary and Garima Singh	Zoology	Journal of Experimental zoology India	2022	0972-0030	https://connectjournals.com/jez

9	79	Coorelation analysis of reactivity in the oxidation of some Aliphatic secondary Alcohols by Imidazalium Dichromate:A Kinetic study	Dr. Deeksha Yajurved i	Chemistry	Journal of Interdisciplinary cycle Research Vol.-XIII (X) pp-329-335	2021	(p) 0022-1945	http://www.jicjournal.com
10	83	Implementation of Genetic Engineering and Novelomics Approaches to Enhance Bioremediation:A focused Review	Dr. Garima Malik	Botany	Bulletin of Environmental Contamination and Toxicology Vol.-182 pp-443-450	2021	0128-021-03218-3	https://www.springer.com/journal/128
11	89	Steady Magneto hydrodynamic Micropolar Fluid Flow and Heat and Mass Transfer in Permeable Channel with Thermal Radiation	Vandana Agarwal	Computer Application	Coatings	2022	2079-6412	https://www.mdpi.com/journal/coatings https://doi.org/10.3390/coatings12010011
12	90	Numerical analysis of heat transfer in magneto hydrodynamic micropolar jeffery fluid flow through porous medium over a stretchingsheet with thermal radiation	Vandana Agarwal	Computer Application	Journal of Thermal Analysis and Calorimetry	2022	1588-2926	https://doi.org/10.1007/s10973-022-11224-8

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You searched for "**Shodh disha**". Total Journals : 1

Search:

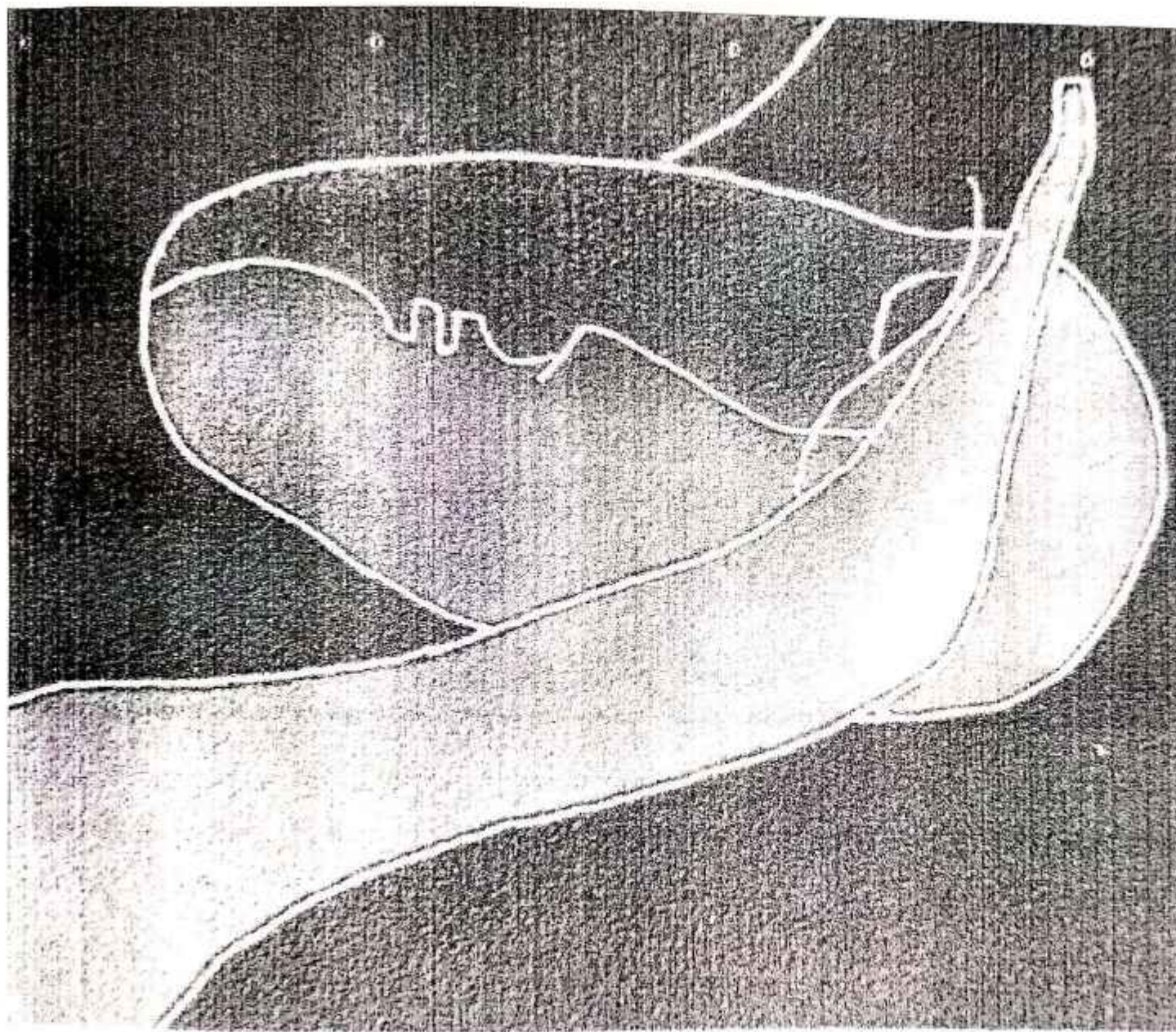
Sr.No.	Journal Title	Publisher	ISSN	E-ISSN	UGC-CARE coverage year	Details
1	Shodh Disha	Hindi Sahitya Niketan	0975-735X	NA	from October - 2020 to Present	View

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प्रकाशन समर्थी से संपादकीय सामग्री आवश्यक नहीं है। पत्रिका से संबंधित सभी विचार केवल विज्ञापन विभाग यापन के अधीन हैं। शुल्क की शर्त 'शोध हिशा' विज्ञापन के नाम पर है। (सन् 1989 से प्रकाशन-क्षेत्र में संचित)

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- डॉ० राजेंद्र मिश्र, 14/4 स्नेहलता गंज, इंदौर 452003 (म०प्र०)
- प्रो० हरिमोहन बुधौलिया, पूर्व आचार्य एवं अध्यक्ष हिंदी अध्ययनशाला, विक्रम विश्वविद्यालय, उज्जैन
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- प्रो० डॉ० सदानंद भौसले, अध्यक्ष हिंदी विभाग, सावित्रीबाई फुले पुणे विश्वविद्यालय, पुणे (मह०)
- प्रो० शंभुनाथ तिवारी, हिंदी विभाग, अलीगढ़ मुस्लिम विश्वविद्यालय, अलीगढ़ (उ०प्र०)
- डॉ० योगेंद्रनाथ शर्मा 'अरुण', (पूर्व प्राचार्य) 74/3 नया नेहरूनगर, रुड़की (उत्तराखंड)
- डॉ० अरविनजेश अक्स्थी, हिंदी विभाग, पी०जी० डी०बी० कॉलेज, नेहरू नगर, नई दिल्ली
- डॉ० अरुणकुमार धगत, अध्यक्ष, मीडिया अध्ययन विभाग, महात्मा गांधी केंद्रीय विश्वविद्यालय, मोतीहाता
- प्रो० मंजुला राणा, अध्यक्ष हिंदी विभाग, हेमवती नंदन बहुगुणा केंद्रीय विश्वविद्यालय, श्रीनगर
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- डॉ० मुकेश गर्ग, पूर्व एसोसिएट प्रोफेसर हिंदी विभाग, दिल्ली विश्वविद्यालय, दिल्ली
- प्रो० जितेंद्र वत्स, प्रोफेसर हिंदी विभाग, माघ विश्वविद्यालय, बोध गया (बिहार)
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- डॉ० दिनशकुमार चौबे, हिंदी विभाग, पूर्वोत्तर पर्वतीय विश्वविद्यालय, शिलांग (मेघालय)
- डॉ० शाहाबुद्दीन शेख, प्राचार्य, लोकसेवा कला व विज्ञान महा०, औरंगाबाद (मह०)
- डॉ० महेशचंद्र, पूर्व एसोसिएट प्रोफेसर हिंदी विभाग, मेरठ कॉलेज, मेरठ (उ०प्र०)
- श्री राकेशकुमार दुबे, पत्रकारिता और जनसंचार विभाग, उड़ीसा केंद्रीय विश्वविद्यालय, कोणार्ड (उड़ीसा)
- डॉ० महेश दिवाकर, अध्यक्ष, अंतर्राष्ट्रीय हिंदी साहित्य एवं कला मंच, मुगादाबाद (उ०प्र०)
- डॉ० प्रणव शर्मा, अध्यक्ष हिंदी विभाग, उपाधि महाविद्यालय, पौलीभीत 262001 उ०प्र०

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प्राप्त करने की चिंता तो करना नहीं चाहते पर यह अवश्य चाहते हैं कि उन्हें साहित्यकार के रूप में आदर मिले, और ख्याति मिले, और प्रशंसा मिले।

प्रकृति ने मनुष्य को जितनी भी शक्तियाँ दी हैं, उनमें सबसे अद्भुत शक्ति कल्पनाशक्ति है। किसी में यह शक्ति कम होती है, किसी में अधिक। जनसामान्य से अधिक कल्पनाशक्ति रखने वाले लोग साहित्य के क्षेत्र में आसानी से सक्रिय हो जाते हैं। किंतु वे इस बात से अवगत नहीं होते कि कला और साहित्य का दुर्ग मात्र कल्पनाशक्ति पर खड़ा नहीं किया जा सकता। कल्पना को ज्ञान, सोच और चिंतन की सामग्री से भी लैस करना होता है। कल्पना के पाँव हवा में होते हैं। उसे ज्ञान और चिंतन ही ठोस धरातल पर स्थापित करता है। ज्ञान अर्जित करने के लिए परिश्रम चाहिए। किंतु प्रशंसा और ख्याति के इच्छुक परिश्रम का कष्ट भोगना आवश्यक नहीं मानते। परिणामतः साहित्य के क्षेत्र में आधे-अधूरे लोगों की भीड़ बढ़ती चली जाती है।

मैं अपने पास आए इस नए कहानीकार से पूछता हूँ कि उसने अब तक किस-किस कहानीकारों को पढ़ा है। उत्तर मिलता है मात्र उन कहानीकारों को जो स्नातक स्तर की पाठ्यपुस्तकों में सम्मिलित रहे हैं। मैं चुप हूँ और कुछ सोचकर फिर एक प्रश्न कर रहा हूँ, 'कहानियों के अतिरिक्त उसने और किस-किस विषय की पुस्तकें पढ़ी हैं। समाज, राजनीति, विज्ञान, मनोविज्ञान, देश और विभिन्न देशों की मानव-सभ्यता से जुड़ा साहित्य।' उत्तर मिलता है, 'नहीं, इनमें से किसी विषय को नहीं पढ़ा।'

मुझे फिर किसी कार्यालय में नौकरी करनेवाला वह क्लर्क याद आ जाता है, जिसकी चर्चा मैंने इस लेख में की है। हमारे इतिहास में 19वीं शताब्दी के उत्तरार्द्ध से बीसवीं शताब्दी की अवधि ऐसी है, जिसमें कार्यालयों, दफ्तरों, फैक्ट्रियों, कल-कारखानों तथा शिक्षण-संस्थाओं में आंशिक दक्षता वाली पीढ़ियाँ तैयार की जाती रही हैं। हिसाब-किताब रखनेवाले क्लर्क के लिए मात्र एकाउंटेंसी का जानकार होना पर्याप्त है। उसे कुछ और जानने की जरूरत नहीं है। कोट सीने वाले टेलर मास्टर को सिर्फ कोट सीने के काम में दक्ष होना चाहिए। उसके लिए कमीज या जैकेट की सिलाई जानना जरूरी नहीं है। पर आंशिक ज्ञान के लोग बाजार और व्यवसाय की दुनिया में तो चल सकते हैं, साहित्य में नहीं चल सकते।

यहाँ मेरा आशय यह बिल्कुल नहीं है कि एक कवि या साहित्यकार को सभी विधाओं का पूर्ण ज्ञान होना चाहिए। मैं यह बिल्कुल नहीं कह रहा हूँ और यह संभव भी नहीं है। मेरे कहने का अधिप्राय केवल इतना है कि साहित्यकार की हैसियत से हमारी जानकारी का क्षेत्र सीमित नहीं होना चाहिए। हमें विभिन्न विषयों के ज्ञानविज्ञान की उतनी जानकारी तो होनी ही चाहिए, जिनसे हमारी सोच और लेखन के क्षितिजों के विस्तृत होने की संभावना होती है।

'कवि (साहित्यकार) उत्पन्न होता है, बनता नहीं।' इस पुरानी कहावत के अर्थों को इतना जड़ और संकुचित मत कीजिए कि यह अंधविश्वास की सीमा में सिमटकर रह जाए। साहित्यकार या कवि उत्पन्न होता है, यह सही है पर इसका अर्थ केवल इतना है कि प्रकृति उसे कुछ ऐसी रचनात्मक प्रवृत्तियाँ देकर संसार में भेजती है जो अन्य सामान्य लोगों में नहीं होती। किंतु प्रकृति द्वारा भेंट की गई इन रचनात्मक प्रवृत्तियों के सहारे कोई भी व्यक्ति साहित्य में कोई बड़ा कारनामा कर दिखाने में सफल नहीं हो सकता, उसे अपनी रचनात्मक क्षमता को ज्ञान, अनुभव, अध्ययन,

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डॉ० सुनीता

एसोसिएट प्रोफेसर

आर०जी० (पीजी) कॉलेज, मेरठ

आज के बदलते सामाजिक संदर्भों और परिस्थितियों ने जीवनमूल्यों और जीवनधारों में अकल्पनीय परिवर्तन कर दिया है। परंपरित जीवन-शैली और जीवनमूल्य आज अयाचित, अर्थहीन, यहाँ तक कि हास्यास्पद से लगने लगे हैं। आज एक जिंदगी को स्वीकार कर उसके लिए जीने-मरने की चाह, निरंतर क्षीण होती हुई उस जड़ावस्था तक पहुँच गई है जहाँ एक नहीं कई-कई जिंदगियाँ हैं, लेकिन कोई भी मन को बाँध नहीं पाती। मन न बाँध सके तो भी जीना एक मजबूरी है, और मजबूरी के बोझ तले घुटते रहना ही आज जिंदगी की नियति बन गई है। युगीन जीवन के विकल्पों की इस वृद्धि ने चुनाव की इन्हीं उलझनों को निरंतर बढ़ाया ही है। चुनाव की इन्हीं उलझनों में फँसा व्यक्ति अपनी सौहार्द व प्रेम की विरासत को निरंतर खोता गया और विरासत की इस गुमशुदगी के साथ-साथ सिमटता गया परिवार का आकार। परिवार के आकार का यह संकुचन एक स्वतंत्र जीवन जीने की आकांक्षा का ही परिणाम था। सीमित आकार वाले इस नए परिवार पर आकर प्रश्न यह उठता है कि जिस स्वतंत्र और निर्बंध जीवन के लिए परिवार को परंपरित परिवार से अलग किया गया, विरासत से विद्रोह किया गया, वह जीवन क्या यह नया परिवार पा सका है? ...नहीं... इस नए परिवार के निर्माता भी कहीं संतुष्ट नहीं हैं? न स्वयं से न एक दूसरे से। एक ही छत के नीचे इनका अजनबीपन इतना बढ़ता जा रहा है कि ये अपने ही घर में अपने आपको नितांत अकेला, अजनबी, मिसफिट और फालतू पाते हैं। संबंधों का यह विखराव और अपनेपन की यह अजनबीयत, नए व्यक्तित्वों को द्वंद्वात्मकता में इस कदर उलझाती जा रही है कि एक किनारे से उन्हें दूसरा किनारा पूर्ण व आकर्षक लगता है लेकिन जब वे दूसरे किनारे पर पहुँचते हैं तो अपूर्णता का अहसास और तीखा हो जाता है तब वे सोचते हैं कि जो पूर्ण था उसे तो वे छोड़ आए हैं जबकि वास्तविकता यह है कि आज अपूर्णता व्यक्ति के मन में घर कर चुकी है किसी इस या उस किनारे में नहीं। पूर्णता की खोज में भटकते अधूरे व्यक्तित्वों के इसी संत्रास का प्रतिबिंब है मोहन राकेश का नाट्य साहित्य। अधूरेपन के अहसास से प्रसित व्यक्तित्वों के द्वंद्व, संत्रास, पीड़ा, छटपटाहट व अकेलेपन को सुदूरगामी ऐतिहासिक व्यक्तित्वों के माध्यम से प्रस्तुत करना राकेश के रचनाकार व्यक्तित्व की महत्वपूर्ण उपलब्धि रही है। 'आषाढ़ का एक दिन' तथा 'लहरों के राजहंस' नाटकों में दो भिन्न ऐतिहासिक चरित्रों के माध्यम से समकालीन द्वंद्व की अभिव्यक्ति के बाद आधे-अधूरे राकेश का पहला ऐसा नाटक है जिसमें उन्होंने पूर्णता की खोज में भटकते अधूरे व्यक्तित्वों से सीधे संपर्क स्थापित किया है, यह संपर्क उनके बाह्य जीवन में उपेक्षित उलझती आंतरिक आवश्यकताओं से। इन आंतरिक आवश्यकताओं की अपूर्णता का अहसास ही द्वंद्व को जन्म देता है और राकेश का रचना-संसार इसी पर आधृत है

'यह कहना गलत नहीं होगा कि राकेश की विशेषता नए-नए महत्वपूर्ण कथानक, व्यापक परिवेश और विविध समस्याओं को देने में उतनी नहीं है जितनी कि आज के मनुष्य के भीतरी दुंद को पकड़ने में।'

'लहरों के राजहंस' और 'आषाढ़ का एक दिन' नाटकों में ऐतिहासिक कथानक के अनुसार तात्कालिक वातावरण को उपस्थिति करने के लिए भाषा व वातावरण में जिस आडंबर का प्रयोग हुआ, 'आधे-अधूरे' उस आडंबर से पूर्णतया मुक्त है। 'लहरों के राजहंस' और 'आषाढ़ का एक दिन' से पूर्णतया भिन्न अभिव्यक्ति आवरण द्वारा समायोजित 'आधे-अधूरे' राकेश का सर्वाधिक चर्चित नाटक है। पूर्व नाटकों से भिन्न वातावरण व भाषा वाले इस नाटक की आरंभिक समीक्षाएँ भी दो अतिवादी छोरों का स्पर्श करती हैं। प्रथम छोर में इसकी अतीव प्रशंसा है तो द्वितीय में इसके महत्त्व को 'एक भावुक नाटक' करार दिया गया है। समीक्षकों के दोनों ही अतिवादी दृष्टिकोण एक मनोवैज्ञानिक तथ्य पर आधारित है जिसके तहत मानव मात्र किसी भी नए विचार या वस्तु को केवल सतही तौर पर देखकर उसे समझे बिना ही उसके प्रति अपनी राय देने की जल्दबाजी करते हैं। इसी मनोवैज्ञानिक पूर्वाग्रह से ग्रसित समीक्षकों ने जब मानवीय दुर्बलताओं व कटु यथार्थ से सीधे संपर्क करने वाले इस नए नाटक को देखा तो दो विपरीत ध्रुव पर झलती समीक्षाएँ कर डाली। समीक्षा के क्षेत्र में यही मानव स्वभाव अक्सर कृति के साथ न्याय नहीं होने देता, क्योंकि अधिकांशतया पाठक या दर्शक वर्ग भी इन प्रारंभिक समीक्षाओं से मस्तिष्क में कृति के प्रति एक पूर्वाग्रह बना लेते हैं और कृति को अपना उचित व संतुलित मूल्यांकन पाने के लिए एक लंबी प्रतीक्षा से गुजरना पड़ता है। कृति की यह प्रतीक्षा दीर्घावधि पश्चात् तब पूर्ण होती है जब इन आरंभिक अतिवादी समीक्षकों से परे कृति का पुनर्मूल्यांकन किया जाता है। 'आधे-अधूरे' नाटक के साथ भी यही सब हुआ और उसे जो निर्विवाद स्थान, प्रकाशन व प्रदर्शन के साथ मिल जाना चाहिए था बहुत बाद में मिल सका।

'आधे अधूरे' नाटक के सभी पात्र इसके शीर्षक का मूर्तिमान रूप प्रतीत होते हैं। इसका कोई भी पात्र अपनी स्थिति से संतुष्ट नहीं है। सभी को सब से शिकायत है, केवल सबसे नहीं स्वयं से भी। राकेश के अन्य नाटकों के समान इस नाटक में भी प्रमुखता पात्रों की ही है कथानक की नहीं। पात्रों में भी इनके पात्र समाज में जीते-जागते, चलते-फिरते व्यक्ति नहीं है अपितु इनके भीतर छिपे अवसाद ग्रस्त व्यक्तित्वों की सजीव सृष्टि है और इसी से ये मंच पर अपनी दृढ़तात्मकता में आम आदमी के उलझाव को व्यक्त करने की पूर्ण सामर्थ्य रखते हैं।

'आधे-अधूरे' नाटक का परिवार मात्र पाँच पात्रों का परिवार है जिसमें स्त्री-पुरुष (सावित्री, महेंद्रनाथ) व तीन बच्चे (बिन्नी, अशोक, किन्नी) हैं। इन पाँचों पात्रों के अतिरिक्त जुनेजा, जगमोहन व सिंघानिया परिवार में कभी-कभी आने वाली अतिथि है। नाटक में आरंभ से अंत तक एक अजीब-सा तनाव व्याप्त रहता है। प्रथम दृश्य में ही जब सावित्री थकी-हारी ऑफिस से घर आती है तो कोई भी सदस्य घर में नहीं मिलता, लेकिन अस्त-व्यस्त सूना, अकेला घर जैसे उसी की प्रतीक्षा कर रहा था। यह घर है जो सबके होने पर बिल्कुल अकेला लगता है और किसी के भी न होने पर अपनी स्थिति से सबके होने का आभास कराता है। इस बिखरे घर के प्रत्येक कोने से घर सदस्यों का बिखरा व्यक्तित्व झाँकता प्रतीत होता है। अशोक की काटी हुई बिखरी तस्वीरें उसकी बेकारी व अन्य दिलचस्पियों की सूचक है, बेकारी की स्थिति में यह अपनी कुछ कर सकने की उम्र को मानो अभिनेत्रियों की तस्वीरों के साथ ही काट देना चाहता है। गृहस्वामी के

सिबल से सजे महेंद्रनाथ के अस्त-व्यस्त पड़े कपड़े उनके व्यक्तित्व के बिखराव की गवाही देते से लगते हैं। और छोटी लड़की किन्नी की फटी किताबें, बिखरा स्कूल बैग उसके जिद्दी व उच्छ्रंखल स्वभाव का परिचय देता है। सावित्री घर को एक मजबूरी के कारण ही सँवारना चाहती है क्योंकि उसका बॉस सिंघानिया घर आने वाला है और घर का बिखराव बाहर नहीं दिखना चाहिए, भले ही घर में कितनी ही घुटन क्यों न घिर जाए। इसी समय महेंद्रनाथ घर में प्रवेश करता है और एक तनाव जो घर में बिखरा था, सिमटकर सावित्री व महेंद्रनाथ के बीच घनीभूत हो जाता है, दोनों एक-दूसरे से जो भी कुछ कहते हैं उससे लगता है मानो वे कहना नहीं लड़ना चाहते हैं। सावित्री को महेंद्रनाथ के प्रत्येक व्यवहार से सख्त शिकायत है तो महेंद्रनाथ भी उसकी प्रत्येक भंगिमा में एक दोष देख ही लेता है।

गृहस्थी के संचालन में महेंद्रनाथ का कोई आर्थिक सहयोग नहीं है। बहुत समय पहले वह अपना काफी धन जुनेजा के साथ मिलकर खो चुका है, तभी से सावित्री की नौकरी से ही गृहस्थी की गाड़ी चल रही है। व्यक्तित्व होने पर भी व्यक्तित्वहीनता का अहसास उसे कभी कटु बना देता है तो कभी अपना ही नाकारा रूप उसे पराजित कर देता है। घर में यह अकेले कोई निर्णय ले सके, क्रोध कर सके या किसी को कोई काम करने से रोक सके, ऐसी उसकी स्थिति नहीं है। अपनी स्थिति को समझकर ही यह सावित्री के गुस्से को स्वीकारता है, कभी स्वयं क्रोधित होता भी है तो एक-दो कटु वाक्य कहकर अपनी स्थिति में लौट आता है। सावित्री शुरू से ही महेंद्रनाथ के व्यक्तित्व में एक अधूरापन महसूस करती रही है। उसे लगता है कि महेंद्रनाथ का अपना कोई व्यक्तित्व नहीं, कि यह अकेले कुछ नहीं कर सकता, कि उसे प्रत्येक स्तर पर एक कसौटी चाहिए, कि वह केवल पानी है जो बार-बार नए बर्तन के साथ नया आकार ले लेता है। 22 सालों के वैवाहिक जीवन में एक आधा-चौथाई आदमी भी सावित्री उसमें नहीं ढूँढ पाती। महेंद्रनाथ के प्रति अपूर्णता की इस भावना को वह आवेशावस्था में जुनेजा के सामने कह देती है— 'और खुद! वह खुद एक पूरे आदमी का आधा-चौथाई भी नहीं है।' वह चाहती है एक पूरा आदमी जो किसी का सहारा ना ढूँढे, अपने आप चल सके और पानी नहीं बर्तन बन सके। महेंद्रनाथ की यह अपूर्णता सावित्री को उससे दूर बहुत दूर करती जाती है और वह पूर्णता की खोज में इधर-उधर भटकती रहती है लेकिन जो अधूरापन महेंद्रनाथ में है वही अधूरापन सावित्री को उन सबमें मिलता है जिन सबमें वह पूर्णता की आकांक्षा रखती थी। खोज की यह अपूर्णता उसमें निरंतर एक खोज भरती जाती है और तब उसे लगता है— 'सबके...सब...सबके सब एक से। बिल्कुल एक से है आप लोग अलग-अलग मुखौटे पर चेहरा?...चेहरा सबका एक ही।' अपूर्णता के अहसास से महेंद्रनाथ भी ग्रस्त है लेकिन फिर भी दोनों साथ हैं, दोनों अपने-अपने स्वभाव से विवश हैं, दोनों स्वयं को नहीं बदल सकते, दोनों एक-दूसरे से नफरत करते हैं फिर भी साथ रहने की विवशता है। अपनी व्यक्तित्वहीनता में महेंद्रनाथ, और बहुत कुछ पाने की चाह में सावित्री, दोनों एक ऐसे मुकाम पर पहुँच चुके हैं जहाँ ये अपने मन की बात नहीं कहते अपितु कुछ भी गलत कहकर एक-दूसरे को अधिक से अधिक दुःखी करना चाहते हैं।

पति-पत्नी के संबंधों की यह दरार बिन्नी, किन्नी व अशोक तीनों को घर से घृणा करने पर मजबूर कर देती है। संबंधों का यह कड़वापन घर को घर नहीं चिड़ियाघर में बदल देता है— 'मैं यहाँ थी तो मुझे कई बार लगता था कि मैं घर में नहीं चिड़ियाघर के एक पिंजरे में रहती हूँ।' इस दमघोंटू पिंजरे से निकलने की इच्छा सब में है और बिन्नी इससे बचने के लिए अपनी माँ के

मित्र मनोज के साथ जाती है, लेकिन घर से निकलकर भी घर के वातावरण से वह मुक्त नहीं हो पाती। नए परिवेश की आवश्यकता नए संबंधों की जिम्मेदारी और पुराने परिवेश के प्रभाव का उलझाव उसे 20 साल की उम्र में 40 साल की प्रौढ़ महिला सा बड़प्पन दे देता है। जिस अकेलेपन और अजनबीयत से बचने के लिए किन्नी घर से भागी थी वही रिक्तता व अजनबीयत यहाँ भी उसे नहीं छोड़ता, उसे लगता है कि 'दो आदमी जितना ज्यादा साथ रहें, एक हवा में सौंसे ले उतना ही ज्यादा अपने को एक-दूसरे से अजनबी महसूस करें।' साथ-साथ रहने से बढ़ने वाला यह अजनबीपन ही है जो आज दांपत्य संबंधों के बिखराव का प्रमुख कारण बनता जा रहा है। पास रहकर भी निरंतर बढ़ने वाली इस दूरी का अहसास ही एक सीमा के बाद स्त्री-पुरुष दोनों को एक नई तलाश के लिए विवश कर देता है। अशोक एक बेरोजगार युवक है जो घर में बाहर की असफलताओं और बाहर घर की बदनामियों से त्रस्त रहता है। ऐसा नहीं है कि यह कुछ करना नहीं चाहता लेकिन उनमें से किसी के माध्यम से यह कुछ नहीं करना चाहता जिनसे सावित्री उसे नौकरी दिलवाना चाहती है। वह उन्हें पसंद नहीं करता, उनके दिखावटी बड़प्पन से चिढ़ता है। उसे लगता है कि जब-जब माँ के ये बॉस घर में आते हैं, तब-तब यह और अधिक बौना हो जाता है—'तो बुलाती क्यों हो ऐसे लोगों को घर पर कि जिनके आने से...कि जिनके आगे हम जितने छोटे हैं, उससे और छोटे हो जाते हैं अपनी नजर में।' अशोक भी समझता है कि यह घर नहीं है और किन्नी यहाँ से प्रेम के कारण नहीं अपितु इस घर से छुटकारा पाने के लिए चली गई है। वह कहीं जा नहीं सकता, यह उसकी विवशता है। स्त्री-पुरुष के संबंधों की कटुता बच्चों के आपसी प्रेम को नष्ट कर उन्हें विद्रोही एवं विषाक्त मानसिकता से ग्रस्त कर देती है। वे सब एक दूसरे से कटे-कटे चिढ़े-चिढ़े से रहते हैं। छोटी लड़की किन्नी मात्र 13 वर्ष की है। अशिष्टता उसके व्यवहार का प्रमुख अंग है उसे भाई-बहन, मम्मी-पापा किसी से प्रेम नहीं उसके मन में है केवल घृणा और विद्रोह। उसके ऐसे व्यवहार का कारण भी घर ही है। घर से बाहर उसे पिता की बेकारी, भाई के नकारा स्वभाव, माँ के पुरुष मित्रों व बहन के घर से भाग जाने को लेकर बहुत सी घृणित बातें सुननी पड़ती है जिससे चिढ़कर वह माँ से उल्टा बोलती है, भाई के साथ अशिष्ट व्यवहार करती है, बड़ी बहन पर हाथ उठाती है और जब कोई उसे समझाता है कि यह बड़ी हो गई है, उसे ऐसा अशिष्ट व्यवहार नहीं करना चाहिए तो बिफर उठती है—'हाँ बड़े हो गए हैं। पता नहीं किस वक्त छोटे हो जाते हैं, किस वक्त बड़े हो जाते हैं।' किशोरावस्था की यह सामान्य समस्या है कि पहले उनकी इस उम्र की जरूरतों पर ध्यान नहीं दिया जाता। फिर उन्हें बड़ों के साथ इसलिए नहीं बैठने दिया जाता क्योंकि वे छोटे हैं और छोटों के साथ इसलिए नहीं खेलने दिया जाता कि ये बड़े हो गए हैं। किशोरावस्था की किन्नी जानना चाहती है कि वह कितनी बड़ी और कितनी छोटी है? यह सवाल किसी का नहीं किशोरावस्था के प्रत्येक बच्चे का सवाल है। कोई इनकी मनःस्थिति को न समझे, इनकी अवहेलना करे तो परिणाम में किन्नी-सा जिद्दी, अभद्र और अशिष्ट व्यवहार आश्चर्य की वस्तु नहीं समझा जाना चाहिए।

आत्मीयता से रहित इस घुटन-भरे वातावरण में भी सावित्री को परिवार की आर्थिक स्थिति सुधारनी ही है। इसीलिए वह सिंघानिया को घर बुलाती है लेकिन बेटे का तिरस्कार उसे हताश व निराश कर देता है। स्थिति जब पूर्णतया असहनीय हो जाती है तो वह उस निर्णय पर पहुँच जाती है जिस पर पहुँचने से सदा डरती रही थी। वह जगमोहन के साथ इस उम्र में नया जीवन बिताना चाहती है लेकिन सफल नहीं हो पाती और हताश होकर जब घर लौटती है, तो घर

में जुनेजा मिलता है. जो उम्मीदें बहुत कुछ या सब कुछ पाने की इच्छा को उसी के सामने अनावृत्त कर देता है—'जिस मुझे मैं तुम कितना कुछ एक साथ भर लेना चाहती थी, उसमें जो था वह भी धीरे-धीरे बाहर फिसलता रह गया।' सावित्री बार-बार जुनेजा की बातों का विरोध करती हुई महेंद्र के साथ रहने से स्पष्ट इंकार कर देती है। लेकिन अंततः उसी मोहरे के साथ जिंदगी बिताने के लिए वह विवश दिखाई देती है जिसे यह बिल्कुल-बिल्कुल नहीं चाहती। दूसरी ओर महेंद्र भी इस घुटन से भागना चाहता है लेकिन भागकर भी भाग नहीं पाता और फिर लौट आता है। दोनों पति-पत्नी के बीच 'एडजस्टमेंट' का अभाव उन दोनों को अपूर्णता में भर देता है। ये दोनों ही अधूरे हैं, दोनों ही किसी दूसरे पूर्ण व्यक्तित्व को खोजना चाहते हैं। इसी पूर्णता की तलाश 'आषाढ़ का एक दिन' में कालिदास व मल्लिका करते हैं और यही पूर्णतानंद सुंदरी या आधे-अधूरे के पात्र, कोई भी इस पूर्णता को न पा सका। कारण...? इस पूर्णता को वे इसी संसार के अपने जैसे ही अपूर्ण व्यक्तित्वों में तलाशते रहे, क्योंकि वे इस शाश्वत सत्य से अनभिज्ञ हैं कि पूर्णता इस संसार में कहीं है ही नहीं और यदि है तो केवल मन की संतुष्टि में।

आधे-अधूरे के पात्रों में अपनी वर्तमान स्थिति से पलायन की जो प्रवृत्ति है, वह केवल उन्हीं की हो या किसी वर्ग विशेष की हो, ऐसा नहीं है। आर्थिक विपन्नता और आकांक्षाओं का अतिशयता में पिसता आज का मानव असफलता के क्षणों में स्वयं को निराश और उदास पाता है। जीवन की निराशात्मक स्थिति ही उसकी अपूर्णता की द्योतक है, उसके अधूरेपन की सूचक है—यह मनःस्थिति जीवन के जिस संकीर्ण वातावरण से जन्म ले रही है, वह अर्थ के साथ जीवन के सांस्कृतिक मूल्यों के हास का ही परिणाम है। सावित्री और महेंद्रनाथ व उनका परिवार परिवर्तित जीवन-मूल्यों के खंडित परिवेश में ही पूर्णता की तलाश का आकांक्षी है। आज संबंधों की डूबती आत्मीयता, अपनों का परायापन और व्यस्तता में गुम होते पहचान के रिश्तों ने एक ऐसा दम घोंटू वातावरण आम-आदमी के चारों तरफ बना दिया है कि वह जहाँ है वहाँ नहीं रहना चाहता है, जबकि यह उसे स्वयं भी ज्ञात नहीं कि वह जाना कहाँ चाहता है? दिशाभ्रम की सी इस स्थिति में वह कहीं-न-कहीं पहुँच जाना चाहता है फिर भले ही यह पहुँचना किसी नए मंजिल रहित मार्ग पर चलने की तैयारी ही क्यों न हो। जहाँ हैं, बस वहाँ नहीं रहना, जो कर रहे हैं, वही नहीं करना, कुछ नया बार-बार नया, हर रोज नया, जब तक जिंदगी है तब तक नया ही नया पाने की यह लालसा सावित्री-महेंद्र मनोज-बिन्नी, अशोक या किन्नी की ही नहीं, अपितु लेखक की अपनी भी है—'जाने क्यों ये बेचैनी मन में बनी रहती है कि यहाँ नहीं रहना है बल्कि जहाँ कहीं भी होऊँ वहाँ नहीं रहना है...आनेवाले कल की शाम यहाँ इस तरह यह करते हुए नहीं काटनी, कहीं और जाकर काटनी है किसी और तरह कुछ और करते हुए।'

नाटक के पात्रों की अजनबीयत भी समकालीन जटिलताओं की जीवन को एक ऐसी देन है जिससे मैं तुम यह वह कोई नहीं बच सकता, जिसे झेलना जरूरी है। एक-दूसरे से चिढ़ते आधे-अधूरे के ये पात्र इतने व्यावहारिक हैं कि इन्हें सड़क चलते किसी भी क ख ग के भीतर ज्यों-का-त्यों पाया जा सकता है। इन पात्रों में आम आदमी का प्रतिबिंब देखकर ही नाटक के निर्देशक ओम शिवपुरी ने अपनी वक्तव्य दिया—'आधे-अधूरे मुझे समकालीन जिंदगी का पहला सार्थक हिंदी नाटक लगता है। यह मौजूदा जीवन की विडंबना के कुछेक सघन बिंदुओं को रेखांकित करता है। इसके पात्र स्थितियाँ एवं मनःस्थितियाँ यथार्थ परक तथा विश्वसनीय हैं।'¹⁰

वास्तव में आधे-अधूरे नाटक अपने संपर्क में आधुनिक मध्यमवर्गीय जीवन की नब्ज पर

अँगुली रखने का अहसास देता है, और साथ ही युगीन संदर्भों में बदलते जीवनमूल्यों और आत्मोद्य संबंधों की औपचारिकता का एक ऐसा विकृत रूप भी सामने रखता है जिसे हम खुद जीते हैं, महसूस करते हैं लेकिन स्वीकारते नहीं। स्वतंत्रता के बाद जो बिखराव मध्यमवर्गीय पारिवारिक जीवन में आया, उसे भले ही मध्यमवर्गीय व्यक्तित्वों द्वारा मुखौटा लगाकर छिपाने का प्रयास किया गया हो लेकिन मुखौटों के भीतर घुटने और सिसकने को मजबूर टूटते आत्मीय संबंधों की इस पीड़ा से बिखरते दांपत्य संबंधों में खोती हुई मासूमियत, भविष्य की तलाश में भटकती बूढ़ी जवानी और अपने-आपसे भी अकेले होते आम आदमी की मजबूरियों को नाटक आधे-अधूरे ने परत-दर-परत इस तरह आवरणहीन किया है कि आधे-अधूरे नाट्य साहित्य की पूर्णता का आदर्श ही नहीं बना अपितु युगीन मध्यमवर्गीय जीवन के संक्रास का सशक्त दस्तावेज भी बन गया।

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डॉ० अरुणकुमार भगत, अध्यक्ष, मीडिया अध्ययन विभाग, महात्मा गांधी केंद्रीय विश्वविद्यालय, मोतीहारी
प्रो० मंजुला राणा, अध्यक्ष हिंदी विभाग, हेमवती नंदन बहुगुणा केंद्रीय विश्वविद्यालय, श्रीनगर
प्रो० हनुमानप्रसाद शुक्ल, हिंदी विभाग, महात्मा गांधी अंतर्राष्ट्रीय हिंदी विश्वविद्यालय, वर्धा
प्रो० चंद्रकांत मिसाल, प्रोफेसर एवं अध्यक्ष हिंदी विभाग, एस०एन०डी०टी० महिला विद्यापीठ, पुणे (महा०)
डॉ० मुकेश गर्ग, पूर्व एसोसिएट प्रोफेसर हिंदी विभाग, दिल्ली विश्वविद्यालय, दिल्ली
प्रो० जितेंद्र खन्स, प्रोफेसर हिंदी विभाग, मगध विश्वविद्यालय, बोध गया (बिहार)
डॉ० माला मिश्रा, पत्रकारिता एवं जनसंचार विभाग, अदिति कॉलेज (दिल्ली विश्व०), बवाना
डॉ० दिनेशकुमार चौबे, हिंदी विभाग, पूर्वोत्तर पर्वतीय विश्वविद्यालय, शिलांग (मेघालय)
डॉ० शहाबुद्दीन शंख, प्राचार्य, लाकसेवा कला व विज्ञान महा०, औरंगाबाद (महा०)
डॉ० महेशचंद्र, पूर्व एसोसिएट प्रोफेसर हिंदी विभाग, मेरठ कॉलेज, मेरठ (उ०प्र०)
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डॉ० पूनम

असिस्टेंट प्रोफेसर, इतिहास विभाग
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भगवान गौतम बुद्ध की जन्मतिथि को लेकर अर्वाचीन पीडितों में बहुत ज्यादा मतभेद है। कुछ का मानना है कि इनका जन्म ईसा पूर्व-486-87वें वर्ष में हुआ। दीपवंश व महावंश के अनुसार बुद्ध का परिनिर्वाण ईसा से पहले 563वें वर्ष में हुआ। यदि इस तिथि को माना जाए तो इस आधार पर उनकी जन्मतिथि ईसा पूर्व 623वें वर्ष में हुई।

भगवान गौतम बुद्ध के बोधिसत्व बनने की प्रथा बहुत प्राचीन है। पालि साहित्य में सबसे प्राचीन 'सुत्रनिपात' है, उसमें भी कहा गया है-

सो बोधिसत्त्वां रतनवरों अतुल्यां।
मनुस्सलोके हितसुखाय जातो
सक्यानं गामें जनपदे लुम्बिनेय्य।

अर्थात् श्रेष्ठ रत्न जैसे बोधिसत्व ने लुम्बिनी जनपद में शाक्यों के गाँव में मानव हित के लिए जन्म लिया है। शाक्यों की राजधानी कपिलवस्तु थी, जिसका नाम महान बुद्धिवादी मुनि कपिल के नाम पर संभवतः पड़ा था।

बोधिसत्व की माँ का नाम महामाया था, इनके बारे में बहुत कम जानकारी मिलती है, परंतु यह बता पाना संभव नहीं है कि शुद्धोधन ने किस उम्र में महामाया से विवाह किया और महामाया ने किस उम्र में बोधिसत्व को जन्म दिया। अपदान ग्रंथ में महा-प्रजापति का एक अपदान है, जिसमें उन्होंने कहा-

पच्छिमं च भवे दानि जाता देवदहे पुरे।
पिता अज्जनसक्को में माता गत्त सुलक्खवा।
ततो कपिल वत्थुस्मिं सुद्धोदनधरं गता।

अर्थात् इसमें बताया गया है कि इस अंतिम जन्म में मैंने देवदह नगर में जन्म लिया। मेरे पिता का नाम अंजन शाक्य और मेरी माता का नाम सुलक्षणा था। इसके पश्चात् मैं कपिलवस्तु के राजा शुद्धोधन के घर गई।

शुद्धोधन बड़ा धनी व्यक्ति था। उसके पास बहुत बड़े-बड़े खेत थे और नौकर चाकर भी अनगिनत थे। कहा जाता है कि उसने अपने खेतों को जोतने के लिए हजार हल रख रखे थे। उसके कई महल थे जहाँ वह बड़े ही आराम से जीवनयापन करता था। सिद्धार्थ गौतम ने शुद्धोधन के घर जन्म लिया। उनके जन्म को लेकर भी रोचक कथा प्रचलित है। महामाया के स्वप्न में सुमंघ नाम का एक बोधिसत्व उनके पास आया और उनसे प्रश्न किया, 'मैंने अपना अंतिम जन्म पृथ्वी पर धारण करने का निश्चय किया है, क्या तुम मेरी माता बनना स्वीकार करोगी? महामाया

का उत्तर था-बड़ी प्रसन्नता से। जब राजा शुद्धोधन ने महामाया के स्वप्न के बारे में पूछा तो उन्होंने भविष्यवाणी की कि 'महाराजा निश्चित रहें। आपके यहाँ एक पुत्र होगा। यदि वह घर में रहेगा तो वह चक्रवर्ती राजा होगा, यदि वह त्याग कर संन्यासी होगा तो वह बुद्ध बनेगा संसार के अधकार का नाश करने वाला।'

जिस समय सिद्धार्थ का जन्म हुआ, उस समय हिमालय में असित नाम के एक बड़े ऋषि निवास करते थे। असित ने सुना कि आकाश स्थित देवता, 'बुद्ध शब्द की घोषणा कर रहे हैं। असित ने देखा कि वह अपने वस्त्रों को ऊपर उछाल-उछाल कर प्रसन्नता के मारे इधर-उधर घूम रहे हैं। उन्होंने सोचा कि मैं उधर क्यों न जाऊँ, जहाँ 'बुद्ध' ने जन्म गृहण किया। जब ऋषि असित ने संपूर्ण जंबूद्वीप पर दृष्टि डाली तो देखा कि राजा शुद्धोधन के घर एक दिव्य पुत्र ने जन्म लिया है। बच्चे को देखने के लिए ऋषि असित राजा शुद्धोधन के महल पहुँच गए और राजा से बच्चे को दिखाने की प्रार्थना की। तब शुद्धोधन बोले, 'ऋषिवर! बालक सोया है। क्या आप थोड़ी देर प्रतीक्षा करने की कृपा करेंगे?' ऋषि का उत्तर था, 'राजन! इस तरह के दिव्य विभूतियाँ देर तक सोती नहीं रहती। वे स्वभाव से ही जागरूक होती हैं।'

असित ऋषि पुरानी भविष्यवाणी से परिचित थे कि यह बच्चा या तो चक्रवर्ती सम्राट बनेगा या फिर महान संन्यासी। असित ऋषि इस बात से भी निश्चित थे कि यह बालक गृहस्थ नहीं बनेगा। बालक को देखकर ऋषि सिसकियाँ लेकर रोने लगे। इस देखकर राजा शुद्धोधन ने उनके रोने का कारण पूछा। इस पर ऋषि असित ने उत्तर दिया, 'मैं जराजोर्ण हूँ, वय प्राप्त हूँ। यह बालक निश्चयात्मक रूप से 'बोधि' लाभ करेगा, सम्यक संबुद्ध होगा। तदनंतर वह लोक कल्याण के लिए अपना धम्म-चक्र प्रवर्तित करेगा, जो इससे पहले इस संसार में कभी प्रवर्तित नहीं हुआ।' आगे असित ऋषि कहते हैं, 'जिस प्रकार राजन! कभी कहीं इस संसार में दुःख पुषित होता है, ठीक उसी प्रकार अनंत युगों में इस संसार में कहीं बुद्धोत्पाद होता है। जो निश्चय ही यह बच्चा बोधि लाभ करेगा, सम्यक संबुद्ध होगा और अनंत प्राणियों को दुःख सागर से पार ले जाएगा और उन्हें सुख प्रदान करेगा। परंतु उस बुद्ध को देखने के लिए मैं यहाँ नहीं रहूँगा। इसलिए राजन! मैं इस दुःख से दुःखी हो रहा हूँ। क्योंकि उस बुद्ध को पृथक् रूप से भाग्य में नहीं बदा है।'

ऋषि असित के साथ उनका भान्जा नाहक भी उपस्थित था। ऋषि असित ने नाहक को सलाह देते हुए कहा, 'नरदत्त! जब कभी तुम्हें वह सुनने का मिले कि वह बालक बुद्ध बनेगा तो जाकर शरण गृहण कर लेना। वह तेरे सुख, कल्याण और प्रसन्नता के लिए होगा।'

जन्म से सातवें दिन महामाया की मृत्यु हो गई। सिद्धार्थ का एक छोटा भाई भी जन्मा नाम 'देव' था। आठ वर्ष की आयु होने पर सिद्धार्थ ने अपनी शिक्षा प्रारंभ की। कि सात वर्षों के उषाके बारे में भविष्यवाणी की थी कि वे ही अष्ट ऋषि उनके आचर्य हूँ। वे बुद्ध भवन के जाते थे, उन्होंने सिद्धार्थ को शिक्षा दिया। इसके सबब सबभित उनके पुत्र मन्वन्त जिन्होंने उनके शरण राज्य की शिक्षा प्रारंभ की। ऋषि मन्वन्त से उन्होंने तब के तब के समाधि करने का मार्ग सीखा।

श्री-श्री सिद्धार्थ का मन एकदम से तन्मय लगा। तबतक सिद्धार्थ को मनसिकी के साथ-साथ साथ ही सिद्धार्थ का जन्म हो निर भी सिद्धार्थ मन के कर्तविक से उनके अन्तर्गत की तन्मय का ही सुख-सुख का शोभा को मन्वन्तों को छोड़ें मन्वन्तों को छोड़ें

Romam

देखकर सिद्धार्थ ने अपने मित्र से कहा, 'एक आदमी दूसरे का शोषण करे, क्या इसे ठीक कहा जाएगा? मजदूर मेहनत करे और मालिक उसकी मजदूरी पर गुलछर उड़ाए यह कैसे ठीक हो सकता है?'¹⁰ सिद्धार्थ को शिकार करने में भी कोई रुचि नहीं थी। उनका कहना था कि 'मैं करते कि एक क्षत्रिय को क्यों लड़ना चाहिए? और गौतमी हमेशा उत्तर देती कि यह क्षत्रियों का धर्म होता है। परंतु सिद्धार्थ उनकी बात से कभी सहमत नहीं होते।'

सिद्धार्थ स्वयं ध्यान लगाते और अपने मित्रों को भी ध्यान लगाने की प्रेरणा देते। परंतु उनके मित्र उनकी बातों पर ध्यान न देकर उन पर हँसते। सिद्धार्थ के पिता को भी उनका ध्यानमुख होना बिल्कुल अच्छा नहीं लगता था। उन्हें लगता था कि यह क्षत्रिय धर्म से बिल्कुल विमुख हैं।

सिद्धार्थ को एकाग्रता से निकालने के लिए राजा शुद्धोधन ने उनका विवाह करने का निर्णय लिया और दंडपाणि नामक शाक्य की कन्या यशोधरा से उनका विवाह करा दिया। हालाँकि दंडपाणि इस बात से बिल्कुल भी खुश नहीं थे क्योंकि उन्हें सिद्धार्थ के 'दांपत्य' जीवन को लेकर संदेह था। विवाह हो जाने के काफी समय बाद यशोधरा ने एक पुत्र को जन्म दिया। जिसका नाम राहुल रखा गया। राजा खुश थे कि उनका बेटा गृहस्थ बन गया किंतु असित ऋषि की भविष्यवाणी भूत की तरह उनका पीछा कर रही थी। शुद्धोधन की आशंका व्यर्थ न थी, उनके भरपूर प्रयास करने के बावजूद भी सिद्धार्थ का मन भौतिकता में नहीं रमता था।

बीस वर्ष की आयु प्राप्त होने पर राजकुमार सिद्धार्थ शाक्य संघ में दीक्षित हुए। जिस समय सिद्धार्थ की आयु 28 वर्ष थी उसी समय रोहिणी नदी के पानी को लेकर शाक्य व कोलियों में संघर्ष शुरू हो गया और दोनों तरफ के किसान हताहत हुए। शाक्यों के सेनापति ने कोलियों के विरुद्ध युद्ध छेड़ने के लिए एक अधिवेशन बुलाया और कोलियों पर आक्रमण करने की अनुमति माँगी। गौतम ने इस प्रस्ताव का विरोध किया और कहा, 'मैं इस प्रस्ताव का विरोध करता हूँ। युद्ध से कभी किसी समस्या का हल नहीं होता। युद्ध छेड़ देने से हमारे उद्देश्य की ही पूर्ति होगी। इससे एक युद्ध का बीजारोपण हो जाएगा।'¹¹ सेनापति ने गौतम की इस बात का विरोध किया और अपने पक्ष में मत माँगे और सेनापति का मत बहुमत से पारित हो गया। दूसरे अधिवेशन में सेनापति ने बीस वर्ष से लेकर पचास वर्ष के शाक्यों के लिए कोलियों के विरुद्ध अनिवार्य सैनिक सेवा की घोषणा की। गौतम ने इस बात का भी विरोध किया परंतु बहुमत के समक्ष उनकी एक न चली और संघ का विरोध करने के चलते उन्हें दंडित किया गया। गौतम के समक्ष तीन विकल्प रखे गए—

1. सेना में भर्ती होकर युद्ध में भाग ले सकते हो।

2. फाँसी पर लटकना या देश से निकलना।

3. अपने परिवार के लोगों का सामाजिक बहिष्कार और उनके खेतों पर कब्जा।

गौतम ने दूसरे प्रस्ताव को स्वीकार किया और देश से निष्कासित होने का फैसला लिया। परंतु कौशल नरेश के भय से उस समय इस प्रस्ताव को स्थगित कर दिया गया। परंतु गौतम अपनी प्रव्रज्या पर अडिग थे। उन्होंने इस संदर्भ में अपने माता-पिता से भी विचार-विमर्श किया। अंत में वे यशोधरा के पास पहुँचे, परंतु कुछ कह न सके। यशोधरा ने ही मौन तोड़ते हुए कहा, 'तुम्हारा निर्णय ठीक है। तुम्हें मेरी अनुमति और समर्थन प्राप्त है। मैं भी तुम्हारे साथ प्रव्रजित हो जाती। यदि मैं नहीं हो रही हूँ तो इसका मात्र यही कारण है कि मुझे राहुल का पालन-पोषण करना है।'¹² 'अब

मैं इतना ही चाहती हूँ कि अपने प्रिय संबंधियों को छोड़-छाड़कर जो तुम प्रव्रजित होने जा रहे हो, तुम किसी ऐसे नए पथ का आविष्कार कर सको जो मानवता के लिए कल्याणकारी हो।' यशोधरा के मुख से ऐसे वचन सुन गौतम बहुत प्रभावित हुए। उन्होंने यशोधरा से राहुल को लाने के लिए कहा और माँ-बेटे दोनों से विदा ली।

ऋषि भारद्वाज ने गौतम के परिव्राजक संबंधी संस्कार पूरे किए और जैसे ही संस्कार पूरे हुए गौतम ने अपनी यात्रा प्रारंभ कर दी। जैसे ही गौतम आश्रम से बाहर आए जनता उनके पीछे-पीछे चलने लगी। यह दृश्य सभी को मोहित किए जा रहा था। उन्होंने कपिलवस्तु से विदा ली और अनामा नदी की तरफ बढ़े। प्रव्रज्या गृहण करते समय गौतम की आयु मात्र 29 वर्ष थी। अनामा नदी के तट तक गौतम को छोड़ने के लिए छन्न उनके साथ गया था। छन्न ने गौतम को समझाने का हरसंभव प्रयास किया परंतु गौतम अपनी बात पर अडिग थे। उन्होंने छन्न को समझाते हुए कहा, 'यदि मैं स्नेह के कारण अपने संबंधियों का परित्याग न भी करूँ तो भी एक न एक दिन मृत्यु हमें एक दूसरे से अनिवार्य रूप से पृथक कर ही देगी।' इसलिए मित्र शोक मत करो और वापस चले जाओ। यदि मन न माने तो जाकर फिर वापस लौट आना।'

कपिलवस्तु से चलकर गौतम राजगृह पहुँचे। यह 400 मील लंबा रास्ता उन्होंने चलकर ही पार किया। उन्हें जो भी रास्ते में मिला, सभी ने उनका अभिनंदन किया।¹⁵ राजगृह पहुँचकर अगले दिन वे भिक्षापात्र हाथ में लेकर भिक्षाटन के लिए नगर में पहुँचे। चारों तरफ से भीड़ ने उन्हें घेर लिया। राजा बिंबिसार ने अपने महल के बाहर लोगों का जमघट देखा तो कारण पूछा। एक दरबारी ने कारण इस प्रकार बताया, 'जिनके बारे में ब्राह्मणों ने भविष्यवाणी की थी कि या तो यह बुद्ध होगा या चक्रवर्ती राजा होगा... यह वही शाक्य पुत्र है जो अब संन्यासी हो गया है। उसी पर लोग नजर गड़ाए हैं।'¹⁶ बिंबिसार के मन में भी गौतम से मिलने का कौतूहल पैदा हुआ और वे अपने कुछ अनुयायियों के साथ गौतम से मिलने पहुँचे। बिंबिसार ने भी उन्हें समझाने का भरसक प्रयत्न किया किंतु गौतम अपने निर्णय से तनिक भी विचलित नहीं हुए। उन्होंने बड़े ही शांत स्वभाव से राजा को उत्तर दिया, 'मैं संसार के कलहों से आहत हूँ। मैं शांति की खोज में हूँ। मैं इस दुख का अंत करने के बदले में इस पृथ्वी का राज्य क्या दिव्यलोक का राज्य भी न चाहूँगा।'¹⁷ गौतम यज्ञों के विरोधी थे। उनका मानना था कि किसी भी भावी फल के लिए किसी निरीह प्राणी की हत्या करना किसी भी कारुणिक शील संपन्न मनुष्य को योग्य नहीं, चाहे फिर उस यज्ञ का फल अनंतकालीन ही क्यों न हो। 'मैं अगले जन्म में मिलने वाले किसी फल की आशा से कोई कर्म करने में प्रेरित नहीं हो सकता, हे राजन् मेरे मन की भावी जन्मों की कल्पना में सुख नहीं मिलता, ऐसे कर्मों की दिशा उसी तरह अनिश्चित और अस्थिर है जैसे बादलों से गिरी वर्षा से प्रताड़ित किसी पौधे की दशा।'¹⁸

जिस समय गौतम राजगृह में कुटी बनाकर रह रहे थे, उसी समय पाँच दूसरे परिव्राजक भी उन्हीं के पास कुटी बनाकर रहने लगे। इनके नाम-कौण्डिन्य, अश्राजित, बाष्प, महानाम तथा भद्रिक थे। वे भी गौतम के व्यक्तित्व से बहुत प्रभावित हुए और उन्होंने बताया कि उनके आने के बाद शाक्यों की औरतों, लड़कों, लड़कियों व आदमियों ने कोलियों के विरुद्ध युद्ध न करने के लिए जुलूस निकाला और शाक्य संघ को अपने निर्णय पर पुनः विचार करना पड़ा। और इस बारे में वे कोलियों से समझौते के पक्ष में थे और नदी के पानी को लेकर दोनों पक्षों में समझौता हो गया। आपका बलिदान व्यर्थ नहीं हुआ। अपनी प्रव्रज्या को लेकर पुनः गौतम ने आत्ममंथन किया कि

जहाँ शाक्यों और कौलियों के बीच युद्ध समाप्त हो गया है तो क्या ऐसी दशा में मेरा संन्यासी बने रहना उचित है। मैं युद्ध समाप्त ही तो चाहता था। या इसके अतिरिक्त और भी मेरा कुछ ध्येय है। उन्हें स्वयं से ही उत्तर मिल गया, क्योंकि समाज में अस्पृश्यता, जातिवाद व्याप्त ही, इन सबको समाप्त कर संसार के कष्टों व दुखों को दूर किया जा सकता है। मुझे इस सामाजिक संघर्ष का समाधान ढूँढ निकालना ही होगा। गौतम ने हर परंपरा हर मत का स्वयं परीक्षण करने का निश्चय किया।

राजगृह छोड़ने के पश्चात् वे ऋषि भृगु के आश्रम पहुँचे। परंतु जब आश्रम में उन्होंने तपस्वियों को अपने शरीर को कष्ट देते हुए देखा तो वे इस पथ से संतुष्ट न हुए। उनका कहना था 'मेरी समझ में तुम्हारा यह तपस्या क्रम नहीं आता, क्योंकि आपकी यह निष्ठा स्वर्ग लाभ के लिए है, किंतु मेरी इच्छा तो यही है कि संसार के दुख के मूल कारण का और उसके दूर करने का उपाय खोज निकाला जाए।' इसके पश्चात् गौतम ऋषि आलार-कालाम के आश्रम पहुँचे और वहाँ सांख्य दर्शन प्राप्त किया।

जिस समय गौतम अपनी समस्या का हल ढूँढ निकालने के लिए नाना तरह के परीक्षण करने में लगे हुए थे, तभी उनके मन में विचार आया कि वे समाधि लगाने का ढंग क्यों न सीख लें। उन्होंने इसके अभ्यास के लिए आलार-कालाम से प्रार्थना की जिसे ऋषि ने बड़ी प्रसन्नता से स्वीकार कर लिया। उन्होंने इस विधि का प्रतिदिन अभ्यास किया। इसके पश्चात् उन्होंने आलार-कालाम के आश्रम से विदा ली। अपनी योग विद्या को चरम तक पहुँचाने के लिए वे ऋषि उदक राम पुत्र के आश्रम पहुँचे और उनके बताए अनुसार योगाभ्यास किया। इसके पश्चात् गौतम मगध पहुँचे।

गौतम ने सांख्य मार्ग व समाधि मार्ग का परीक्षण कर लिया था। परंतु ऋषि भृगु के आश्रम से वे तपचर्या का परीक्षण किए बिना ही वापस आ गए थे। इसलिए उन्होंने तपचर्या का परीक्षण करने का निश्चय किया। इसके परीक्षण के लिए उन्होंने नेरंजरा नदी के तट पर एकांत स्थान को चुना। उरुवेला में उन्हें पुनः वे पाँच परिव्राजक मिले, और उन्होंने बताया कि वे भी तपचर्या का अभ्यास कर रहे हैं। उन तपस्वियों ने गौतम से प्रार्थना की कि वे उन्हें भी अपने साथ लेकर चलें। गौतम ने स्वीकार कर लिया। गौतम ने तपस्या के लिए कठोर रास्ता अपनाया, वे दिन में कम से कम भोजन गृहण करते। गौतम की यह कठोर तपस्या छः वर्ष तक जारी रही। उनका शरीर इतना कमजोर हो गया कि वे हिल-डुल भी नहीं सकते थे। कठोर तपस्या से उन्हें कोई नया प्रकाश नहीं दिखाई दिया, और संसार में जो दुख की समस्या है, और जिस पर उनका मन केंद्रित था, उस समस्या का कोई हल उसे नहीं दिखाई दिया।¹⁹ उन्होंने मन में सोचा, 'यह न आत्मा विजय का मार्ग है, न पूर्ण बोधि प्राप्त करने का मार्ग है और न मोक्ष का मार्ग है।'²⁰ 'मैं पूछता हूँ क्या शरीर का अधिक से अधिक उत्पादन 'धर्म' हो सकता है?' जिसके शरीर का बल जाता रहा, जो भूख तथा प्यास से परेशान है जिसका मन थकावट के मारे एकाग्र और शांत नहीं है—ऐसे आदमी को कभी नया-ज्ञान प्राप्त नहीं हो सकता।²¹ यही कारण था कि गौतम ने सुजाता द्वारा दी गई खीर को स्वीकार कर लिया और इस प्रकार उनकी कठोर तपस्या का अंत हुआ। उनके पाँचों शिष्यों ने इस बात से रुष्ट होकर उनका साथ छोड़ दिया।

गौतम अब इस निष्कर्ष पर पहुँचे कि पूर्व में उन्होंने जितने भी मार्ग अपनाए वे विफल रहे। परंतु उन्हें इस बात का विश्वास था कि उन्हें रास्ता अवश्य मिलकर रहेगा। सुजाता द्वारा दी गई

खीर ग्रहण करने के पश्चात उन्हें पाँच स्वप्न आए। उन्होंने अपने सपनों की यही व्याख्या की कि उसे 'बोधि' प्राप्त होकर रहेगी। आशा से भरपूर होकर उन्होंने उरुवेला छोड़ दिया और पूर्व दिशा की ओर बढ़ने का विचार किया। रास्ते में उन्होंने पीपल का वृक्ष देखा। उस पीपल के वृक्ष के नीचे गौतम सीधा पद्यासन लगाकर बैठे। और उन्होंने दृढ़ संकल्प किया, 'चाहे मेरी त्वचा, नसों और हड्डियाँ ही बाकी रह जाएँ, चाहे मेरा मांस और रक्त शरीर में ही सूख जाए, बिना 'बोधि' प्राप्त किए मैं इस स्थान का परित्याग नहीं करूँगा।'¹²

जिस समय गौतम दृढ़ आसन लगाकर बैठे थे तो बुरे विचारों और बुरी चेतनाओं के झुंड़ ने उस पर आक्रमण किया। गौतम को डर लगा कि कहीं ये उस पर काबू न कर ले परंतु वे दृढ़ होकर समाधि लगाकर बैठे रहे। ध्यान करने के समय के लिए गौतम ने इतना भोजन इकट्ठा करके पास रख लिया था कि चालीस दिन तक कमी न पड़े। ज्ञान प्राप्ति के लिए गौतम को चार सप्ताह एक लगातार ध्यान-भग्न रहना पड़ा। गौतम को अंतिम अवस्था तक पहुँचने के लिए चार सौद्विगुण पार करनी पड़ीं। चौथे सप्ताह के अंतिम दिन उनका पथ कुछ प्रकाशित हुआ। उन्हें दिखाई पड़ने लगा कि उनके समझ दो समस्याएँ हैं—पहली यह है कि संसार में दुख है और दूसरी यह कि दुखों का अंत किया जा सकता है और मानव जाति को सुखी बनाया जा सकता है।¹³

इस प्रकार चार सप्ताह तक लगातार चिंतन करने के उपरांत अंधकार समाप्त हुआ और नया ज्ञान प्रकाशित हुआ। अविद्या का नाश हुआ और ज्ञान अस्तित्व में आया, उसे एक नया पथ दिखाई दिया। जिस समय गौतम ध्यान में बैठे हुए थे उस समय सांख्य दर्शन का बड़ा प्रभाव था। यह सर्वविदित था कि संसार में कष्ट और दुख है। गौतम का ध्येय यह पता लगाना था कि इन दुखों को किस प्रकार दूर किया जाए। उन्होंने स्वयं से प्रश्न किया कि वे कौन से कारक हैं जिनके चलते मनुष्य कष्ट उठाता है? उनका दूसरा प्रश्न था कि दुख का नाश कैसे किया जाए? इन दोनों प्रश्नों का उन्हें सही-सही उत्तर मिल गया... यही सम्यक संबोधि कहलाया। जिस पीपल के वृक्ष के नीचे बैठकर उन्होंने ज्ञान प्राप्त किया वह वृक्ष-बोधि वृक्ष कहलाया।¹⁴ सम्यक संबोधि प्राप्त कर गौतम-बोधिसत्व बन गए।

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IMMUNOSTIMULATORY EFFECT OF ASCORBIC ACID, ALPHA TOCOPHEROL AND THEIR COMBINATION ON ASIAN CATFISH, *CLARIAS BATRACHUS* (LINN. 1758)

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ABSTRACT : In the present investigation, ascorbic acid, alpha-tocopherol and their combination were used as an immunostimulant on Asian catfish, *Clarias batrachus*. For the present experimentation fishes of average length 20 ± 5 cm and weight 150 ± 200 g were used. Fishes were divided into 4 groups namely I, II, III and IV. Fishes of group I were kept as control, Group II were administered with different doses of ascorbic acid, group III were administered with different doses of Alpha-tocopherol and group IV were administered with the dose of combination of ascorbic acid and alpha-tocopherol. Macrophage phagocytic activity is considered to play a significant role in assessing the non-specific immunity in fishes. The effect of these vitamins and their combination were analysed on the basis of macrophage percentage phagocytosis and macrophage phagocytic index. The phagocytic assay were studied on 5th, 7th and 14th days. A positive correlation between ascorbic acid, alpha-tocopherol and their combination and phagocytic assay were observed as compared to control.

Key words : Ascorbic acid, alpha-tocopherol, *Clarias batrachus*.

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INTRODUCTION

Aquaculture, one of the fastest growing food producing sectors, is gaining momentum in several parts of the world (Labh and Shakya, 2014). Aquaculture is also called underwater agriculture (Khan *et al*, 2011). Several commercial fish species have been cultured intensively in narrow or enclosed spaces such as ponds, cages or tanks under overcrowding or high density conditions, thereby causing adverse effect on their health with a potentially stressful environmental and infectious disease (Jadhav *et al*, 2006). These occurrences have spread through the uncontrolled movement of live aquatic animals resulting in the transfer of pathogenic organism among countries (Lunden and Bylund, 2000). Unfortunately, antibiotics treatment is not successful and sustainable due to increase antibiotic-resistant in bacteria, negative effects on the indigenous microflora of juvenile or adult fish (Misra *et al*, 2006). The manipulation and control of fish health and production by natural substances has been identified as an important area for future developments in aquaculture. Immunostimulants are

dietary additives, which enhance the innate (non-specific) defence mechanisms and increase resistance to specific pathogens. Immunostimulants can be grouped under chemical agents, bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines (Sakai, 1999). Immunostimulants are chemical substances which activate leucocytes (Lunden and Bylund, 2000). Some vitamins can also be used as immunostimulants. Some major vitamins like A, B, C, D and E have been reported to boost up the immune system by strengthening the activity of immune cells during any pathogen attack or in the case of the intrusion of some toxic materials either inhaled from air or that may be present in the foods (Aslam *et al*, 2017). Vitamins have the properties to help the body in fighting against a variety of illness and protect the body from damage cells. Many foods contain vitamin that promote, enhance and regulate the immune system (Aslam *et al*, 2017). Jain *et al* (2012, 2015, 2016) also worked on natural immunostimulants like garlic, vitamin C and *Curcuma longa*. Vitamin E and ascorbic acid work closely together in stimulating the function of the immune system (Romach *et al*, 1993).

Aquatic animals fed with high doses of vitamin C exhibit improved immunity and resistance to disease (Tewary and Patra, 2008). Vitamin C plays a major role in activating and maintaining the function of phagocytes (Pardue and Thaxton, 1984).

The appropriate dose of vitamin E can enhance the generation of antibodies and complement activity in response of antigens, promote the proliferation and differentiation of lymphocytes and cytokine production and improve cytotoxicity and phagocytosis (Villegas *et al.*, 2006).

MATERIALS AND METHODS

Experimental fishes

Catfish, *Clarias batrachus* with average weight of 150 ± 200 g and 20 ± 5 cm length were procured from local fish market, Meerut, U.P., India. They were kept under observation for injury and disease conditions, and only healthy fishes were used for this study. After washing with 0.01% KMnO_4 solution for 15 min, they were placed in glass aquarium (100 L) containing non-chlorinated water. Prior to start the experiment, the fishes were acclimatized to the food and laboratory conditions.

Experimental design

After acclimatization, fishes were divided into 4 groups, each group contained 10-12 fishes, and groups were named as Group I, Group II, Group III and Group IV.

Group I : Control, injected with double distill water.

Group II : Injected with 0.5 mg and 1 mg of Ascorbic acid. The dose of Ascorbic acid more than 1 mg were also injected, but survival was very poor, it was not tolerated by fishes.

Group III : Injected with 0.45 mg, 0.9mg, 1.35mg and 1.8mg of Alpha-tocopherol.

Group IV : Injected with combination of Ascorbic acid and Alpha-tocopherol (0.5mg+0.45mg).

These dose were given intraperitoneally to the respective groups. These treated fishes from different groups sacrificed on 5th, 7th and 14th day after the dose were given.

Percentage phagocytosis & phagocytic index

The fish splenic macrophage monolayer was prepared according to the method of Namaware *et al.* (1994).

For this fishes were anaesthetized, spleen was taken out and passed through nylon mesh into PBS. Splenic cell suspension was collected and centrifuged for 15 min. Supernatant was discarded and pellet was resuspended

in 10 ml DDW and PBS (10x) for RBC lysis. This suspension was centrifuged at 1500 rpm for 15 min and supernatant was discarded and pellet was suspended in RPMI-1640. 200 μ l of cell suspension was plated on each pre-washed slide and incubated in CO₂ incubator for 2-4hrs. Slides were washed with PBS to remove non-adherent cell, 90% of the adherent cell population was of macrophages. Then macrophage monolayer was flooded with 200 μ l of yeast cell suspension and phagocytosis was allowed to occur in CO₂ incubator at 25°C for 90 min. Then slides were rinsed with PBS to remove extra yeast cells. After this slides were fixed in methanol for 15 min and stained with Giemsa for 20 min. Slides were washed with methanol to remove extra stain. Slides were then air-dried and mounted in DPX.

% phagocytosis = No. of macrophages showing phagocytosis/100 macrophages

Phagocytic index = % phagocytosis \times Mean no. of yeast cell per phagocytic macrophage.

OBSERVATIONS AND RESULTS

The data obtained from the above experiment were subjected to statistical analysis using software SPSSv16.0. The result indicated that % phagocytosis and phagocytic index increased significantly ($P \leq 0.05$) in all the groups and on all the days tested as compared to control.

In the Group II : fishes treated with Vitamin C, highest % phagocytosis response was seen with a dose of 1.0 mg/100gm. Body weight on the 5th day and highest phagocytic index was seen with a dose of 1.0 mg/100gm. Body weight on the 5th day.

In the Group II : Fishes treated with Vitamin E, highest % phagocytic response was seen with a dose of 1.35 mg/100gm. Body weight on the 14th day and highest phagocytic index was seen with a dose of 0.9 mg/100 body weight on the 7th day.

In the Group IV : Fishes treated with combination of ascorbic acid and alpha-tocopherol, highest % phagocytosis and phagocytic index was seen on 7th day.

DISCUSSION

Various immunostimulants are now being added to fish feeds and vaccines. In some cases they are injected alone at points of time near predictable fish stressors. Immunostimulants are effective in reducing mortalities in farmed fish. This study has shown that all the non-specific immune parameters of immunocompetence were affected by intraperitoneal injection of the above said immuostimulants.

Macrophages are considered to be the principle phagocytic cell population in fish and the production of

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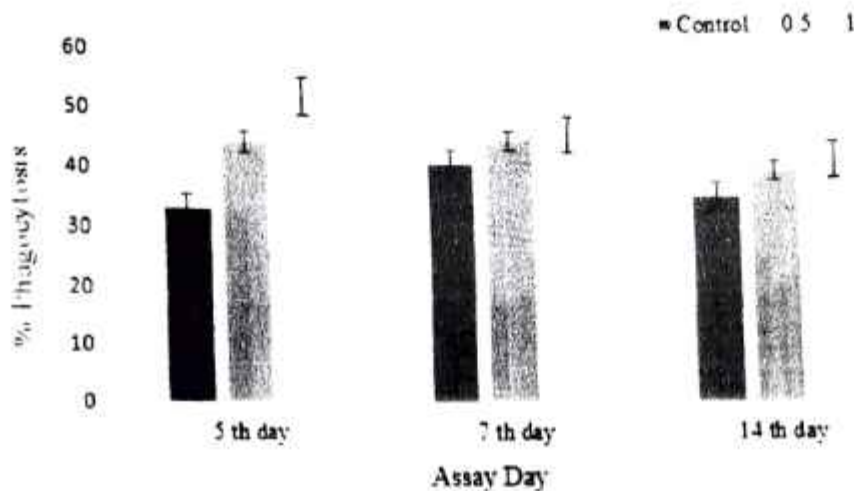


Fig. 1 (a) : Percentage Phagocytosis observed on various assay days after intraperitoneal injection of Ascorbic acid in normal healthy *Clarias batrachus*. Data are expressed as mean \pm S.D. Mean values with different superscripts are significantly different ($P \leq 0.05$).

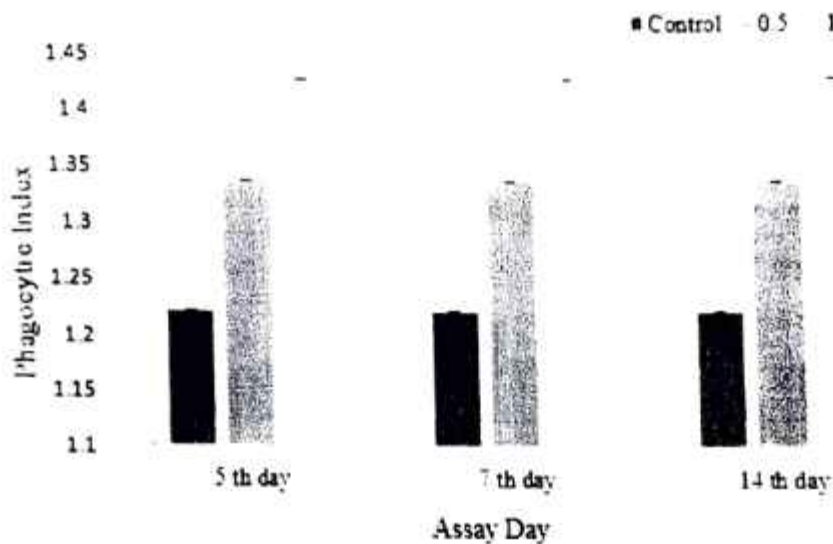


Fig. 1 (b) : Phagocytic index observed on various assay days after intraperitoneal injection of Ascorbic acid in normal healthy *Clarias batrachus*. Data are expressed as mean \pm S.D. Mean values with different superscripts are significantly different ($P \leq 0.05$).

the reactive oxygen intermediates, a major antimicrobial mechanism (Adams and Hamilton, 1992) is also well established in fish phagocytosis. The present study indicates that both percentage phagocytosis and phagocytic index increased significantly ($P \leq 0.05$) in all the immunostimulant injected groups at all sampling times as compared with the control.

The role of vitamin C in enhancing the phagocytic response has been reported by several authors (Thomas and Holt, 1978). The phagocytic index of peritoneal macrophages has been shown to be significantly lower

in rainbow trout fed with a vitamin C deficient diet (Blazer, 1982). Ascorbic acid deficiency also significantly reduced phagocytosis of *Edwardsiella ictaluri* by channel catfish neutrophils (Li and Lovell, 1985). No work has been published on the effects of vitamin C on Phagocytic Spleen Cells. Jain and Varma (2015), as published their work on Immunomodulation induced by vitamin C on healthy and immunocompromised Indian snake head *Channa punctatus* (BI).

As our similar results were also been observed by Waagbo (1994), according to him vitamin E protects

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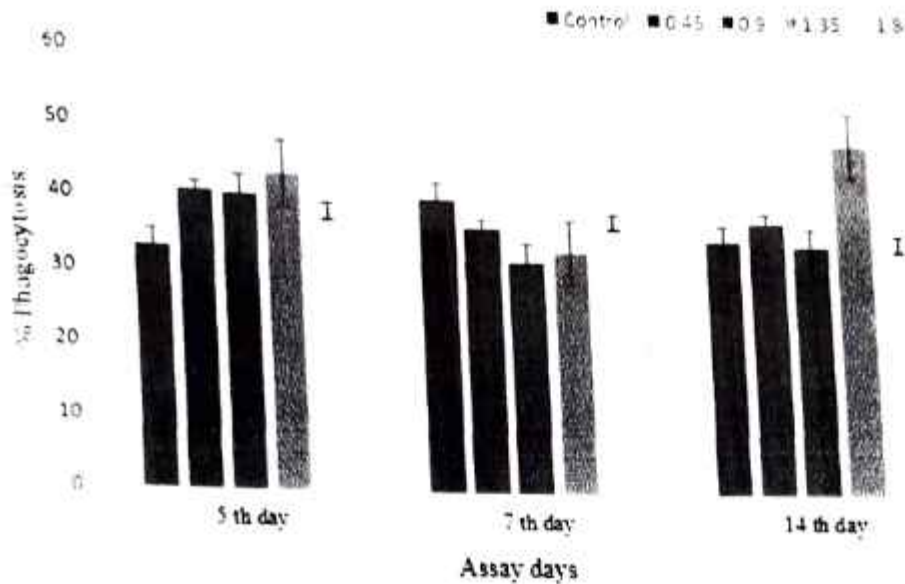


Fig. 2 (a) : Percentage Phagocytosis observed on various assay days after intraperitoneal injection of Alpha-tocopherol in normal healthy *Clarias batrachus*. Data are expressed as mean \pm S.D. Mean values with different superscripts are significantly different ($P \leq 0.05$).

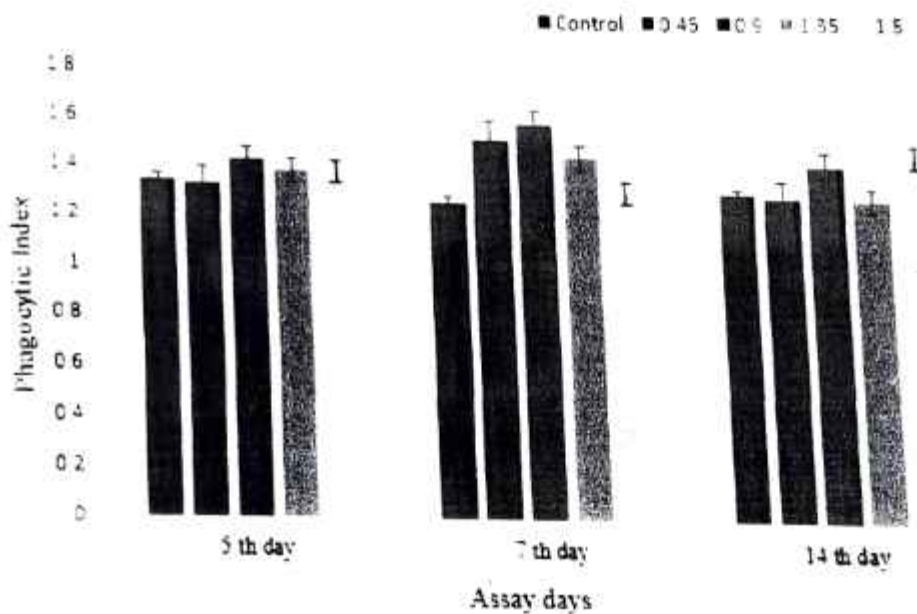


Fig. 2 (b) : Phagocytic index observed on various assay days after intraperitoneal injection of Alpha-tocopherol in normal healthy *Clarias batrachus*. Data are expressed as mean \pm S.D. Mean values with different superscripts are significantly different ($P \leq 0.05$).

macrophage membranes from peroxidative damage by free radicals and thus has key role in the fish immunity. Supplemental vitamin E enhances antibody production against pathogens by promoting increased proliferation

of antibody-producing cell (Tengerdy *et al.*, 1973). Vitamin E plays a crucial role in regulating and supporting immune system function as a potent antioxidant (Jaya-Wardena *et al.*, 2020).

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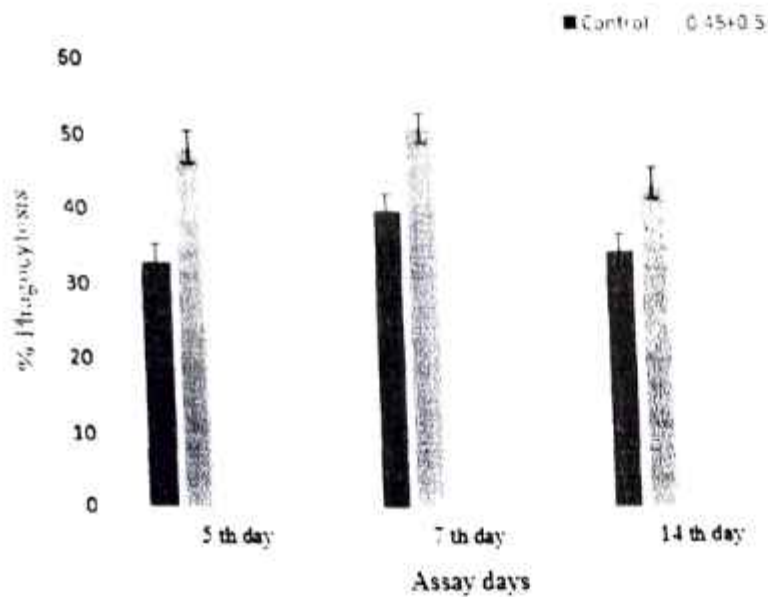


Fig. 3 (a) : Percentage Phagocytosis observed on various assay days after intraperitoneal injection of combination of ascorbic acid and alpha-tocopherol in normal healthy *Clarias batrachus*. Data are expressed as mean \pm S.D. Mean values with different superscripts are significantly different ($P \leq 0.05$).

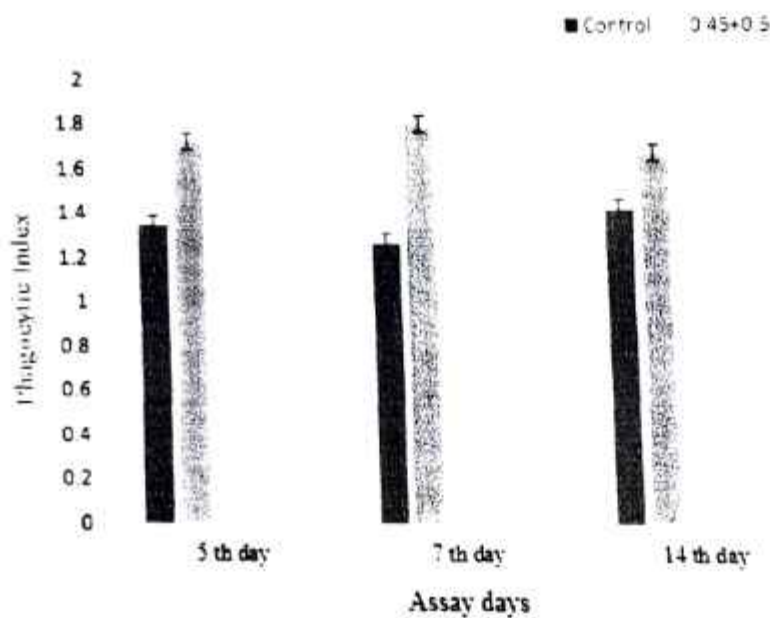


Fig. 3 (b) : Phagocytic index observed on various assay days after intraperitoneal injection of combination of ascorbic acid and alpha-tocopherol in normal healthy *Clarias batrachus*. Data are expressed as mean \pm S.D. Mean values with different superscripts are significantly different ($P \leq 0.05$).

The result presented in this paper demonstrated that ascorbic acid, alpha-tocopherol and their combination were able to produce a significant immunostimulatory

effect in Asian catfish, *Clarias batrachus*.

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REVIEW ARTICLE

Immunomodulatory Effect of Vitamin C and E on Non-Specific Immune Parameters in fishes: A Review

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ABSTRACT

Aquaculture has been fastest growing industry for several decades that include fish and shellfish production. Culture of fishes in narrow space or uncontrolled production becomes the reason of various infectious diseases in fishes. So, prevention from diseases and increasing immunity is important for healthy production. There are many ways in which immunity may be enhanced like use of antibiotics and vaccines in fishes. The use of antibiotics is not very successful and vaccination is an expensive method, so there is need of eco-friendly and less expensive method. Immunostimulants are chemical compounds that activate the innate or non-specific immune system of fishes by activating the cells of immune system. Several vitamins like vitamin E (Alpha-Tocopherol) and vitamin C (Ascorbic Acid) act as an immunostimulant. Vitamin E is a fat soluble compound that enhances the growth, reproduction and survival by increasing the non-specific immunity. It also acts as an antioxidant by controlling the production of free-radicals. Vitamin C is also essential for fish growth, health and survival. It enhances the phagocytosis, serum haemolytic activity, complement activity and proliferation of immune cells. In past few years, stabled beneficial effects of immunostimulants in fishes promoted their application for disease management in aquaculture practice. As we have taken up similar study on the effects of vitamins as natural immunostimulants on Asian, catfish *Clarias batrachus*, so, this review is an attempt to throw light on the role of Vitamin C and Vitamin E as an immunostimulants in fish culture.

1. Introduction

Aquaculture is flourishing sector with various resources and potential and is very important economic activity including fish culture. India ranks second in aquaculture and third in fish production, 1.07% to the National GDP and 5.30% to the agriculture GDP. Due to intensification of culture practices in aquaculture, diseases caused by microbes have surfaced significantly in culture systems. Several drugs, synthetic chemicals and

vaccination programmes have been practiced to prevent and control the diseases, but partial successes have been achieved. An alternative approach has been the application of various compounds to boost or stimulate the immune system (Sakai, 1999). Immune system is a form of protection consisting of thymus, spleen, lymph nodes and some specific immune cells (Coico, 2009). Cells involving the immune system are leucocytes or white blood cells those can be found in the blood stream or on tissue (Fernandez, 2002).

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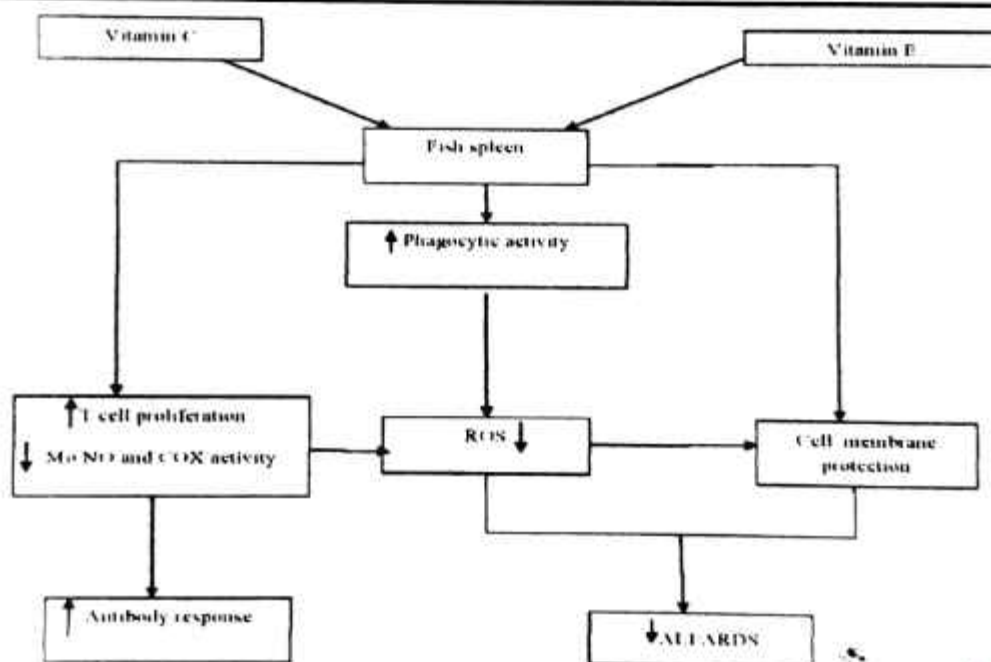
The cells that stimulate the Immune system are macrophages and neutrophils which enhance the non-specific immunity of the organism. Many of these substances adhere to the surface of phagocytes and lymphocytes and stimulate the production of Interferon, interleukin and sophisticated compositions, consequently activating the immune system (Bulut, 1998). The alternative techniques to prevent the diseases has been proposed by strengthening of fish immune system through the application of immunostimulants, immunomodulation by contrast is a consequence of a change in the number or function of the cells involved in the immune response. The most proven effect of immunostimulants is to facilitate the function of phagocytic cells and increase their bactericidal and fungicidal activities (Sakai, 1999).

For the last twenty years, the problem of microbial diseases has emerged as a major constraint to aquaculture industry. Increased disease occurrences have resulted due to the transfer of pathogenic organism among cultured species of fish and shrimp, between different countries without proper quarantine measures. (Sakai, 1999). Fish like Ayu, Carp, Catfish, tilapia and Salmonids are often kept at high population densities. This increases the risk for dramatic disease outbreaks. Although antibiotics can be used for the treatment of bacterial diseases, repeated use can induce drug resistance in microorganisms or suppress the

Immune system of fish (Rijkers et al., 1980b). Unfortunately, antibiotics treatment is not successful and sustainable due to increase antibiotic-resistant in bacteria, negative effects on indigenous microflora of juveniles or adult fish (Misra, 2006). Accumulation of antibiotics residues in fish tissue and environment causes various health issues in human and animal. Vaccination is expensive and stressful to the fishes. A single vaccine is effective against only one specific type of pathogen, but limits the effectiveness for wide range of pathogens due to the complex antigenic structure. (Ardo et al., 2008). Therefore, eco-friendly disease-preventive alternative techniques have to be taken into account. One such promising alternative technique to strengthen fish immune system is the application of immunostimulants in aquaculture (Jadhav et al., 2006). This study will give a hand to fish feed industry and farmers in the formulation of nutritionally balanced practical feed with and optimum dose of natural immunostimulants to enhance the immunity of fishes against various diseases.

2. Immunostimulants

An immunostimulant is defined as chemical, drug, stressor or action that enhances the innate or non-specific immune response by interacting directly with cells of the immune system and activating them. Immunostimulants can be grouped under chemical agents, bacterial preparations,



Vitamin Activity in Fish

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polysaccharides, animal or plant extracts, nutritional factors and cytokines (Sakai, 1999). Fish and shrimp depends more heavily on non-specific defence mechanisms than mammals and therefore immunostimulants play a vital role in health management strategies of aquatic organisms. There are at least 20 different compounds, including levamisole, lypopolysaccharides, β -glucan, Vitamin C and E etc. that are used as immunostimulants, adjuvants and vaccine carriers in fishes (Anderson, 1992). Among the vitamins, the antioxidants (Vitamin C and E) are found to have an impact on the immune system of fishes (Furanose et al., 1992 and Verlhac et al., 1996).

The use of immunostimulants can protect fish from several infectious diseases and decrease mortality rates by increasing fish resistance against bacteria such as *Vibrio anguillarum*, *V. salmonicida*, *Aeromonas salmonicida* and *Streptococcus sp.* Viral infections such as IHN (Infectious Hematopoietic Necrosis) and Yellow-head (YHV) disease and parasitic infections such as white spot disease and sea lice, but immunostimulants do not increase resistance against *Renibacterium salmoninarum*, *Pseudomonas piscida* or *Edwardsiella ictaluri* infections due to their resistance to phagocytosis and abilities to survive within macrophages (Perazzolo, 1997). Anumohini (2011) studied the immunomodulatory effect of β -Glucan on some non-specific immune parameters in *Channa punctatus*. Jain and Varma (2012) observed the effects of Garlic on non-specific immune parameters in *C. punctatus* infected with *Aeromonas hydrophilla*. Jain (2015) studied the immunomodulatory effects of natural immunostimulants on non-specific immune parameters in *C. punctatus*. According to their work they suggested that intraperitoneal injection of these immunostimulants (*viz.* Garlic, Vitamin C, Turmeric and β -glucan) are able to modulate the non-specific immunity in *C. punctatus* without accompanying undesirable side effects. All the non-specific immune parameters *viz.* Phagocytic assay, Bactericidal activity, Lysozyme activity and Superoxide anion production were enhanced significantly on all the days and with all the doses as compared to control. Jain et al., (2015) studied the immunomodulation induced by vitamin C on healthy and immunocompromised Indian snake head, *C. punctatus*. Vitamins are vital organic nutrients in our meal that utilized by the cells of our body for proper growth, development, disease prevention like deficiency disorders, and to

improve immune system efficacy. Vitamins are taken routinely along with diet and are required in proper trace amounts so that all the barriers of the immune system such skin, cell-mediated and humoral immune response etc. could be sustained (Ibrahim, 2015). Vitamins are organic compounds Jain and Varma (2016) observed the effects of *Curcuma longa* on non-specific immunological profile of Indian snake head, *C. punctatus* infected with *A. hydrophilla*. Vitamins are organic compounds that are essential for life they are required in trace amounts for normal growth, reproduction and health (Gasco et al., 2018).

3. Role of Vitamin C (Ascorbic Acid) In Fishes

Ascorbic acid is water soluble vitamin and plays a key role in various metabolic function and physiological function. There is a decrease in absorption of calcium by gill, skin, bone and muscle in fish when there is Ascorbic Acid deficiency in the diet (Pang, 1971; Mahajan and Agrawal, 1980 a and b). Durve and Lovell (1982) found that a dietary supplementation of 30 mg vitamin C/kg supported normal growth and prevented deficiency signs in channel catfish. Li & Lovell (1985) treated Channel Catfish with 300 mg of Ascorbic Acid per kg feed and observed enhanced antibody response, phagocytic ability of potential macrophages complement activity and survival after infection *Edwardsiella tarda* and *E. ictaluri*.

Whli et al., (1986) studied the effect of dietary Ascorbic Acid on the disease resistance of rainbow trout infected with the holotrichous ciliate, *Ichthyophthirius multifiliis*. Most animal can synthesise vitamin C in sufficient quantity for normal growth and function, but a few, such as primates, guinea pigs, some birds and many fishes cannot because they lack the enzyme L-gulonolactone oxidase for synthesis of Vitamin C from glucose (Dabrowski, 1990). Vitamin C is an essential nutrient in aqua-feeds and is an indispensable nutrient required to maintain physiological process such as normal growth, immunity and reproduction of different animals including fishes (Teshima et al., 1991). Hardie et al., (1991) found that serum complement activity of Atlantic salmon was significantly reduced in AA-depleted fish. In their study they found that on bath challenge with a virulent strain of *A. salmonicida*, a significant increase in mortality was seen in AA depleted fishes. According to Dunagan & Lovell (1994), channel catfish showed maximum survival and antibody production and minimum blood

abnormalities when ascorbic and folic acids were incorporated in their diet. Vitamin C is essential for collagen formation, wound healing, haematopoiesis and detoxification of compounds as well as for several metabolic functions including as part of the antioxidant system. In recent years, considerable research has been directed to determine the role of Vitamin C in the immune function and disease resistance in fish. High dose of vitamin C increases the resistance to several bacterial and viral pathogens in fish (Waagbo, 1997; Verlhac and Gabauda, 1997). Vitamin C has been shown to stimulate serum haemolytic complement activity, proliferation of immune cells, phagocytosis, the release of signal substance and antibody production (Verlhac and Gabauda, 1997). More recently, research with Atlantic salmon (*Salmo salar*) has indicated that Vitamin C may protect fish against Vitamin E deficiency in a dose-deficient manner (Hamre, 1997).

Fish lack L-gluconolactone oxidase enzyme, which is responsible for the de novo synthesis of vitamin C, and therefore, they fulfil their Vitamin C requirements from exogenous sources (Fracalossi et al., 2001). Vitamin C facilitates the absorption of iron and is necessary for a maximum rate of immune responses and enables a good response to stressors. Fish showed deficiency sign if they were fed diets deficient in Vitamin C (Roberts, 2001). Vitamin C is considered to be an essential component in diets for teleost fish (Haliver 1985; Dabrowski and Ciereszko, 2001). Vitamin C has a distinct role as a cofactor for enzymes engaged in hydroxylation of proline and lysine, and is required for synthesis of collagen and construction of bone matrix (Dabrowski, 2004). It was reported that dietary Vitamin C enhanced the growth in channel catfish, *Ictalurus punctatus* (Duncan and Lovell, 1997) and even in Indian major carp, *L. rohita* (Tewary and Patra, 2008; Misra et al., 2007). L-ascorbic acid phosphate (ROVIMIX®STAY – C®35) is a stable form of Vitamin C which used in feed and several studies have reported the use of this form of Vitamin C in juvenile Mexican silverside, *Menidia aestivalis* (Martinez-Palacios et al., 2007), Juvenile yellow catfish, *Pelteobagrus fulvidraco* Richardson (Liang et al., 2007a), *Sparus aurata* (Amerio et al., 2000), and Nile tilapia, *Oreochromis niloticus* (Barros et al., 2014). In several fish species, dietary Vitamin C required for maximum body growth exceeds the basic requirement level for growth, survival and hydroxyproline concentrations (NRC, 2011).

Enhancement of growth rate and weight gain observed in Nile tilapia upon vitamin C supplementation could be ascribed to the vitamin C induced stimulation of protein synthesis (Chages and Val, 2003; Faramarzi, 2012). Dietary inclusion of vitamin C for enhancing fish resistance against bacterial infection has also been reported for Nile tilapia and Juvenile Cobia, *Rachycentron canadum* (Khalil and El Hardy, 2015; Zhou et al., 2012). According to Jain et al., (2015) lysozyme activity and bactericidal activity significantly increased with the vitamin C treatment in *C. punctatus*. Highest response was seen with Vitamin C dose of 1.0 mg/100gm. body weight on the 5th day in normal healthy fishes and in immunocompromised fishes the response was seen with a dose of 1.5mg/100gm body weight on the 7th day. The use of Vitamin C alone or in combination with *Echinacea purpura* (EP) successfully enhanced the immune parameters in Nile tilapia, *Oreochromis niloticus* (Abdel Rahman et al., 2018b).

4. Role of Vitamin E (α -tocopherol) in fishes

Supplemental vitamin E enhanced antibody production against a variety of particulate or soluble antigens by promoting increased proliferation of antibody-producing cells (Tengerdy et al., 1973) and by stimulating the response to T-cell mitogens as well as the mixed lymphocyte response to murine spleen cells (Corwin & Gordon, 1982). The survival in eyeing stage of the fertilized eggs gets reduced when Ayu (*P. altevilis*) fed diets with low α -tocopherol levels (Takeuchi et al., 1981).

Vitamin E (α -Tocopherol) is another important micronutrient that affects the reproductive performance of fishes. Increasing vitamin E in the diet increases spawning success, egg survival, hatchability and larval survival of Ayu (*P. altevilis*), red sea bream (*Pagrus australis*), increase the gonado-somatic index and vitellogenesis of common carp (*Cyprinus carpio*) (Watanabe and Takashima, 1977; Kanazawa, 1985). Vitamin E deficiencies in trout result in reduced protection against *Y. ruckeri* (Blazer and Wolke, 1984). Blazer & Wolke (1984) showed that both T-cell (migration inhibition factors) and B-cell (Plaque forming cell)-mediated responses were compromised in rainbow trout, *Oncorhynchus mykiss* which fed on low Vitamin E diets. Gupta et al., (1987) observed higher gonadosomatic index, bigger ova and complete spawning in three major carps (*L. rohita*, *Catla catla* and *C. carpio*) by adding vitamin E in

5. Future Aspects

In present scenario, increasing use of chemical antibiotics in fish culture may cause various health problems in fishes as they become resistant against the antibiotics and reduce the growth rate and delayed reproductive cycle. The chemical antibiotics accumulate in fishes and can be transferred to human population via food chain. To avoid these kinds of problems there is need to replace these chemical antibiotics with harmless, easily available and eco-friendly alternatives. In this reference, Vitamin C and Vitamin E can be used as immunostimulants to increase the quality of fish food in aquaculture. Vitamin C helps in growth of healthy brooders, improve hatchability and proper egg and sperm formation and Vitamin E enhances specific and cell-mediated immunity in fishes. It is believed that in coming years with reference to aquaculture, natural immunostimulants will find more exercise to make aquaculture sustainable and may be an effective tool for controlling infectious diseases in fish food industry.

6. Conclusion

The use of immunostimulants can control the fish and shellfish from several disease and mortality in aquaculture. Vitamins are organic compounds that also can act as immunostimulators like vitamin C and E. Vitamin C (Ascorbic acid) is an essential nutrient which helps in normal growth, immunity and reproduction of fishes. Vitamin E (α -tocopherol) enhanced antibody production against various antigens. It is essential for growth, antioxidant capacity, immunity and reproduction in fishes. In our study, the effect of Vitamin C and Vitamin E and their combination was observed and increase in macrophage activity was observed in all three cases on *C. batrachus was done* (Singh and Jain, 2022).

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THERAPEUTIC ROLE OF *SPIRULINA PLATENSIS* AGAINST CADMIUM SULPHATE TOXICITY IN THE HISTOPATHOLOGY OF LIVER OF *CLARIAS BATRACHUS* (LINN.)

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ABSTRACT: *Spirulina platensis* is a cyanobacteria which is used as a health product have many important nutrient used as a chelating agent to reduce the toxic effect of the heavy metal from the fish. *Clarias batrachus* is an important food fish in India affected by the cadmium contamination of the water resources. Our study is based to observe the protective role of *Spirulina platensis* in the histological alteration in the liver of *Clarias batrachus* affected by Cadmium sulphate toxicity. During this experiment the fishes were divided into three groups, the first group served as control, group II treated with 1.39mg/l cadmium sulphate along with basal diet and group III fed with *Spirulina platensis* supplemented diet along with cadmium sulphate for 30 and 45 days. After 30 days duration of time the liver of fishes of group II shows various histopathological alteration such as cytoplasmic vacuolisation with desheaped hepatocytes with enucleation and these alteration were more severe in 45 days in the liver and showed pyknosis, karyorrhexis, necrosis, cellular degeneration of the hepatocytes with dilation of sinusoids and blood vessels. When *Spirulina platensis* supplemented diet feed the fishes along with cadmium sulphate in group III it reduce the toxic effect of the cadmium and showed less histopathological alteration as in the fish liver as compared to cadmium treated group II.

Key words: *Spirulina*, cadmium sulphate, *Clarias batrachus*.

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INTRODUCTION

Water pollution caused by heavy metal is considered as one of the major problems of the world even in India also (Eliezabby, 2001). Heavy metal such as Ni, Hg, As, Cr, Pb, Cd contaminated the water source through various natural and anthropogenic activities. The contamination of water through heavy metal by various methods has become a threat not only for fish but also for public health, because fish is the main source of the food. Heavy metal bioaccumulate in the fish and transfer in the human population in the form of food by the process of biomagnification. In all among the heavy metal Cd related contamination of the aquatic ecosystem has greatly increased in the last few decades, an increase of Cd deposition in the tissue of aquatic organism in all food chain system (Giles, 1988). It enters the environment as a contaminant by various anthropogenic activities such as mining and metallurgy, sulphate fertilizer, scrap metal

treatment and through chemical industrial activities as electroplating and batteries manufacture (Cherin and Goyer, 1989). These sources of cadmium contaminate the sources of aquatic ecosystem and affect the health of the fish.

Clarias batrachus is an important food fish of India also affected by the cadmium pollution. Cd accumulates in the liver of fish through the digestion process and causes many histopathological alterations in the liver and reduces the function of metabolic processes. To control this problem it is important to alleviate the heavy metal pollution in the aquatic ecosystem through using suitable or low cost antidotes such as using *Spirulina*.

Spirulina platensis is a cyanobacteria used in many countries as a health product for human and animal also because it has many nutritional supplements to improve the health of human, animals and fishes. The nutritional component of *Spirulina* contains up to 70% of protein, 20

% carbohydrate, lipid 7%, mineral, Vitamins, and various important phytochemical which neutralized and detoxify the organism caused by heavy metal (Pintero *et al.*, 2001; Chamaroo *et al.*, 2002; Sharma and Jha, 2010; Jha *et al.*, 2012). The protective effect of *Spirulina platensis* against the cadmium induced toxicity and also is attribute to its antioxidant and chelating effect (Wu *et al.*, 2005).

This study was aimed to find out the protective property of *Spirulina platensis* against Cadmium sulphate toxicity in the liver of catfish *Clarias batrachus*, histopathologically and observed the potential role of *Spirulina platensis* as antioxidant against liver toxicity of fish.

MATERIALS AND METHODS

Experimental fish and their maintenance

A total number of 90 healthy fishes *Clarias batrachus* collected from local fish market of Meerut district with average length of 15 ± 2 cm and average weight upto 60 ± 8 gm. Collected fishes immediately wash for 5 minutes 0.01% $KMnO_4$ to avoid any dermal infection. After washing fishes were acclimatized for 15 days in laboratory condition using tap water in glass aquarium. Fifty percent of water was changed daily during acclimatization. The water quality of aquarium was monitored (pH- 7.2 ± 0.06 , Temperature - 23 ± 2).

Calculation of LC_{50} of cadmium sulphate

Acclimatized fishes were exposed to different concentration of cadmium sulphate (2, 4, 6, 8, 10, 12, 14 mg/l) for determination of LC_{50} value. Cumulative mortality will be determined after 96 hours. The dead fishes will be removed once they will be observed. LC_{50} value will be determined by probit analysis by 96 hours median tolerance limits (Litchfield and wilcoxon 1949) LC_{50} value of cadmium sulphate for *Clarias batrachus* was 1.39mg/l.

Experimental design

Acclimatized and healthy 90 fishes were taken out for the experiment and divided into equal three groups of 30 specimen fishes. The group I of fishes were used as a control, group II of fishes was treated cadmium sulphate with basal diet and the group III of fishes was treated cadmium sulphate with *Spirulina platensis* supplemented diet 10% of their body weight. The water was changed every second day and the fishes were feed daily twice a day. After 30 and 45 days of exposure time the fishes were removed from all the three groups and they were scarified by decapitation and liver was removed for histopathological studies. Histopathological section immediately fixed in 10% formalin solution and

histopathological slide was prepared. The histopathological study was carried out after 30 and 45 days exposure time. The histopathological slide was observed under an Olympus research microscope and photograph was taken and analysed the difference between among the all three groups.

Preparation of diet

Acclimatized fishes were fed with basal diet (contained 35% protein) 10% of their body weight twice a day (Sharma and Jha, 2010). Basal diet was prepared using fish meal (51.25%), wheat flour (36.75%) Cod liver oil (10.00) and minerals (2%). While supplemented diet was prepared replacing same quantity of wheat flour with *Spirulina platensis* as described by James *et al.* (2009).

Diet composition (% dry weight)

Ingredients	Basal diet	<i>Spirulina</i> supplemented diet
Fish meal	51.25	51.25
Wheat Flour	36.75	26.75
Cod liver oil	10.00	10.00
<i>Spirulina</i>	-	10.00
Mineral mix	2.00	2.00
Total	100.00	100.00

RESULTS AND OBSERVATION

Liver of a fish is a glandular structure, which is composed of polygonal cells have a central granular and deeply stained nucleus. It play major role in detoxification and elimination of toxic substance from the body. Histopathological section of the group I control fish showed normal histoarchetctural structure as normal polygonal shaped hepatocytes, granulated cytoplasm and uniform nuclei (Fig. 1). Histopathological alteration in the liver of fish is depends upon the dose and time of exposure. After 30 days exposure of time the liver of fish of group II showed many histopathological alteration as loosening of hepatic tissue, cytoplasmic vacuolisation, deshaped nuclei with enucleation (Fig. 2), while these alteration were more severe after 45 days in this group and showed nuclear degeneration, pyknosis and karyorrhexis along with the hypertrophy of hepatocytes (Fig. 3).

Spirulina platensis showed the recovery of the cells of the liver of the group III fishes and the histpathological alteration were less severe in this group as compared to group II (Fig. 4). Cytoplasmic vacuolisation and enucleation were less seen after 30 days with less degenerative hepatocytes. *Spirulina platensis* also reduced the pyknosis, Karyorrhexis and nuclear degeneration along with hypertrophy of hepatocytes after 60 days as compared to group II (Fig. 5).

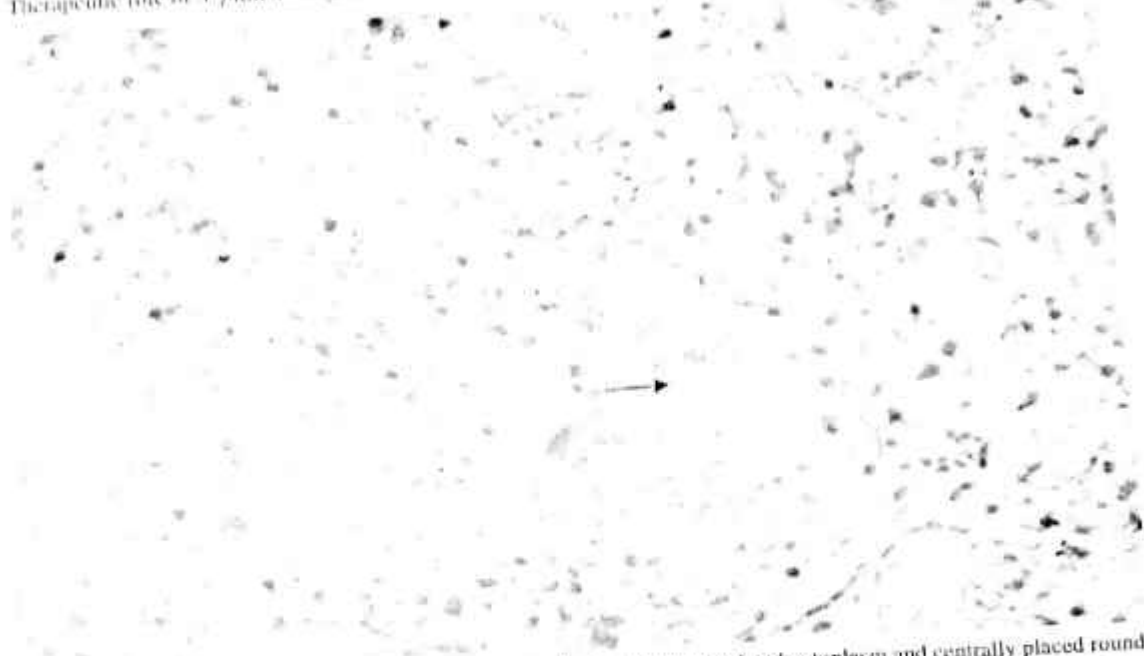


Fig. 1 : Liver structure of control fish showing normal hepatocytes (→) with granulated cytoplasm and centrally placed round nuclei.

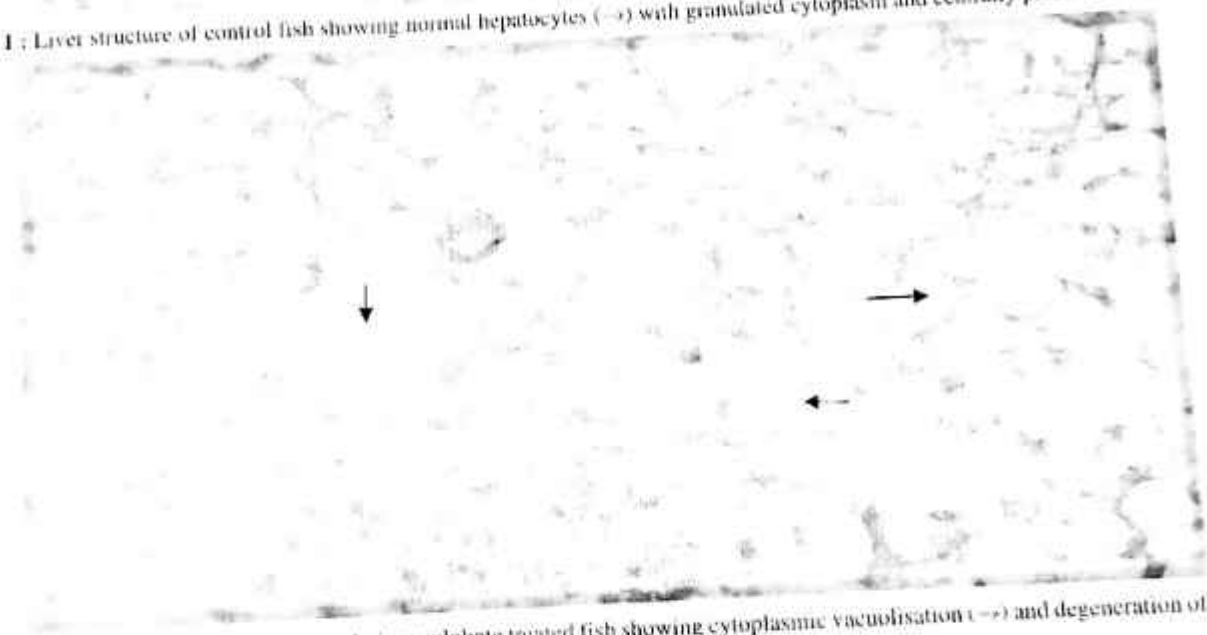


Fig. 2 : Liver structure of after 30 days cadmium sulphate treated fish showing cytoplasmic vacuolisation (→) and degeneration of cells (↓) with enucleation (←).

DISCUSSION

In our study, liver of fish *Clarias batrachus* shows normal histoarchetectoral structure of control group with no pathological alternation. Liver of fish group II which is treated with Cadmium sulphate shows severe histopathological alteration during exposure of time after 30 and 45 days. The section of liver of fish showed cytoplasmic vacuolization enucleation, cellular degeneration, Pyknosis, Karyorrhexis and dilation of sinusoids along hypertrophy of Hepatocytes. These

histopathological alternation shows that the abnormal condition of liver which affect the function of liver and reduced the metabolic process and cause the health of the fish. Our findings were similar to Rani and Ramanmurthi (1989), who observed the effect of Cadmium Chloride at 5 and 50 ppm after 1, 7, 15 and 30 days in *Tilapia mossambica*. Vacuolization in hepatocytes with mild hepatocellular necrosis and shrinkage of hepatocytes in the liver of *Clarias batrachus* after exposure of mercury and cadmium observed by Salvanathan (2013). Dyk *et al* (2007) reported that the

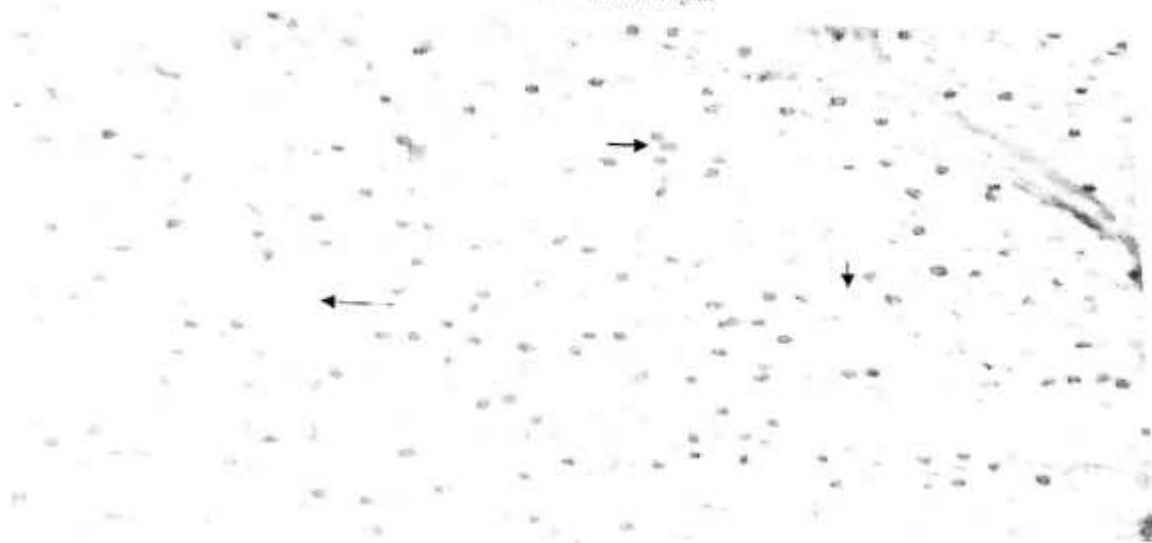


Fig. 3: Liver structure of after 45 days cadmium sulphate treated fish showing cytoplasmic vacuolisation (←) and degeneration of cells (↓) with enucleation, pyknosis (→), hypertrophy of hepatocytes.

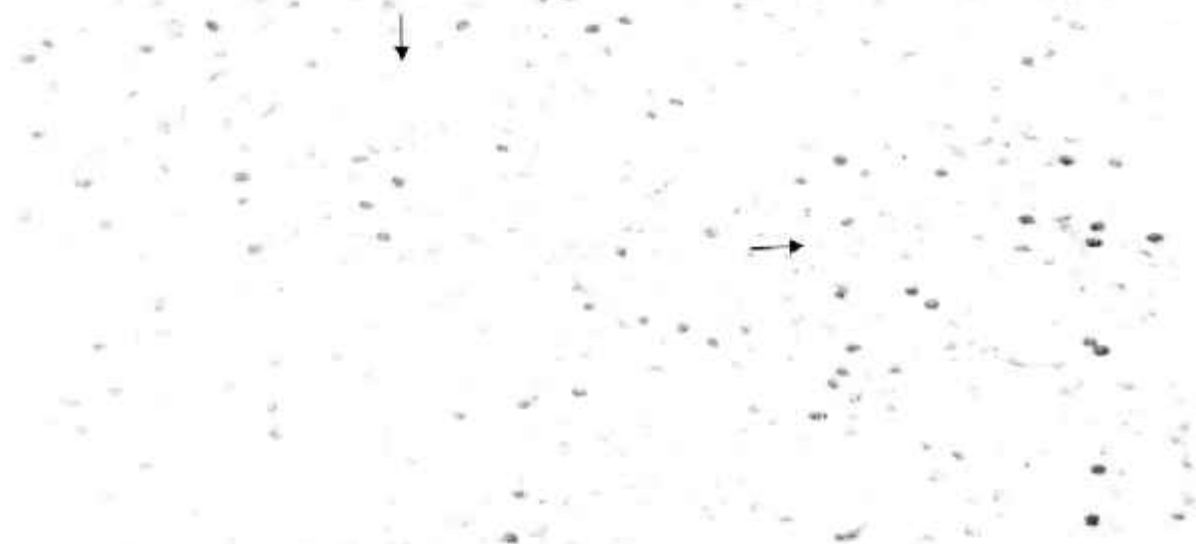


Fig. 4: Liver structure of after 30 days fed with *Spirulina* supplemented diet and cadmium sulphate treated fish showing less cytoplasmic vacuolisation and compactness of hepatic tissue (→) with more or less normal (↓) shape.

swelling in the liver of cells and vacuolization of hepatocytes after exposure of Cadmium in *Oreochromis mossambicus*.

Vacuolization of Hepatocytes caused by the inhibition of protein synthesis, disaggregation of microtubules or shifts substrate utilisation caused energy depletion. (Hinton and Lauren, 1990). Denaturation of volume regulating ATPase or disruption of cellular energy transfer processes required for ionic regulation caused the swelling in the cells (Hinton and Lauren, 1990). Morsey and Protasowicki (1990) observed the effect of Cadmium in the liver of *Cyprinus carpio* and reported that Cd caused atrophy and necrosis of hepatic cells which decreases the size of nuclei and nucleoli and indistinguishable cell

membrane in the liver. Cd caused oxidative stress by several pathway through inhibit the mitochondrial electron-transport chain reaction, leading to accumulation of semi ubiquitous toxicant which enable and to form superoxide redicals (Wang *et al*, 2004).

Our findings showed that *Spirulina platensis* play important role to reduce the toxic effect of Cadmium in the liver of *Clarias batrachus* and finding were similar to Bilal *et al* (2014) who observed the protective role of *Spirulina platensis* against Cadmium chloride in the liver of freshwater fish *Clarias batrachus* and proved that *Spirulina platensis* supplemented diet reduce the histoarchetectural alteration in the liver of fish. *Spirulina platensis* also reduce the loosing of hepatic tissue.



Fig. 5: Liver structure of after 45 days fed with *Spirulina* supplemented diet and cadmium sulphate. Top and fish showing less cytoplasmic vacuolisation (←), enucleation, compactness of hepatic tissue (→) with centrally placed nuclei (↓) and less hyperplasia of cells.

vacuolated cell cytoplasm, enucleation and eccentric nuclei in the 0.6 mg/l mercuric chloride effected catfish *Clarias batrachus*. It is proved that an extract of *Spirulina platensis* significantly reduces the mercury induced hepatotoxicity (Saroch *et al.*, 2012). The chelating property of *Spirulina* may be due to the presence of β carotene (Prescott, 1978; Seshadhi and Jeeji Bai, 1992) vitamin C and E and Phycocyanin (Mathew *et al.*, 1995 and Shimamtsu, 1989). The antioxidant property of *Spirulina* is due to the presence of phycocyanin (Bernyo *et al.*, 2008). β carotene of *Spirulina* may reduce the cell damage and playing the role in the repair of regeneration process of damaged cell (Foote *et al.*, 1970; Gerster, 1993 and Lexia *et al.*, 1996). *Spirulina platensis* used as most of the scientist suggest that 3-20% *Spirulina* in the diet used as a protective agent against metal toxicity in the fishes (Bermejo *et al.*, 2008; Islam *et al.*, 2008; James *et al.*, 2009; Sharma and Jha, 2010). In our study the fishes of group III showed less histopathological alteration as compared to group II, fishes feed with *Spirulina* supplemented diet along with Cadmium show less several histopathological alteration in the liver of *Clarias batrachus*. Cellular vacuolation, pyknosis dilation of sinusoids and cellular generation reduced after 30 and 45 days as compared to cadmium treated group II.

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PROTECTIVE EFFECT OF *SPIRULINA PLATENSIS* IN THE HISTOPATHOLOGICAL ALTERATION IN THE KIDNEY OF *CLARIAS BATRACHUS* AGAINST CADMIUM SULPHATE TOXICITY

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ABSTRACT: Increased industrialisation practices has raised the level pollution of Cadmium Sulphate in the aquatic ecosystem. *Clarias batrachus* is an important food fish which is greatly affected by this pollution and it causes various pathological alteration in the fish and affect the nutritious value of the fish. This study is based on the hepatoprotective role of *Spirulina platensis* in the kidney of *Clarias batrachus* against Cadmium Sulphate after 30 and 45 days interval of time. *Spirulina platensis* is a well known cyanobacteria having good nutritious value and used to detoxify the heavy metal from the body of fish. In this experiment, the fishes were divided into 3 groups. First group served as control shows normal histoarchitectural structure of kidney. In group II which is treated with Cadmium Sulphate (1.39 mg/l) showed various histopathological alteration in the kidney after 30 day. It showed tubular and haemopoietic tissue vacuolisation and necrosis with widened bowmen's spaces with deslumped glomerulus and these symptoms were more severe with time i.e. after 45 days. In group III fishes treated with Cadmium Sulphate alongwith *Spirulina platensis* diet, the kidney of fish showed better result and the histopathological lesion were less severe as compared to group II after 30 and 45 days interval of time.

Key words: *Clarias batrachus*, *Spirulina platensis*, kidney, cadmium sulphate

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INTRODUCTION

The contamination of freshwater through heavy metal has devastating effect on the aquatic life. Among all the heavy metal contamination, Cadmium is the most common and toxic element, which can be accumulated, stored and assimilated by the aquatic organism through the food chain resulting in the pathological alteration in the organism and reducing the growth rate and also affect the reproductive cycle. (Pigott and Tucker, 1990; Ruter, 1995). Pollution of Cadmium because of high anthropogenic activities such as mining, electroplating of metals, batteries and plastic industries industries has caused great threat to aquatic life because of their bioaccumulation potentials (Nsofor *et al.*, 2014). Cadmium pollution in the aquatic ecosystem has greatly affected the food fishes such as *Clarias batrachus* and caused histopathological alteration in the organs of the fishes.

Clarias batrachus is an important food fish which is contaminated by various heavy metals and suffers from various pathological alterations with consequent inhibition

of metabolic processes, alterations in Haematological parameters and damage to liver, kidney, gills and other organs. Reynders *et al.* (2008) reported that the tissue level Cadmium concentration highest in the kidney of *Cyprinus carpio*. Cadmium accumulates in the tissue of carps in the following order Kidney > Gill > Liver.

Spirulina platensis is cyanobacterium, which is well known for its protective effect against heavy metal toxicity (Amin *et al.*, 2006). It can be used to detoxify heavy metal from water and food and may also be used to chelate or detoxify and neutralize the poisonous effect of heavy metals from water, food and environment. *Spirulina platensis* provides phycocyanin, a source of biliverdin which is among the most potent of all intra-cellular antioxidants (Bangeppagari *et al.*, 2014). The metalloprotective role of *Spirulina* may be due to the presence of β -carotene (Seshadri, 1991). *Spirulina* have rich content of vitamin C, E and β -carotene and this phytochemical constituent may reduce the cadmium toxicity and enhance the radical scavenging property

(Bangeppanagi, 2014). *Spirulina platensis* is rich in proteins, carbohydrates, polyunsaturated fatty acids, Sterols, minerals and vitamins (Piterra *et al.*, 2001; Chamorro *et al.*, 2002; Sharma and Jha, 2010; Jha *et al.*, 2012). The general composition of *Spirulina platensis* is protein 69%, carbohydrate 20%, lipids 7%, minerals 5% and the phytopigments are beta carotenoids, xanthophylls, zeaxanthin, phycocyanin and also contain various vitamins such as B₁, B₂, B₆, B₁₂, Folic acid, Inositol, vitamin K and also including minerals as Iron, Calcium, Magnesium, Manganese, Potassium, Zinc and Selenium so because of its nutritional value, it is used as a dietary supplement for astronauts on space missions. NASA has stated that the nutritional value of 1000 kg of fruits and vegetables equals one kg of *Spirulina* (Ravi *et al.*, 2002).

This study was aimed to find out the protective role of *Spirulina platensis* against Cadmium Sulphate toxicity in the kidney of catfish, *Clarias batrachus*, histopathologically and observed the potential and therapeutic role of *Spirulina platensis* as an antioxidant against kidney toxicity of fish.

MATERIALS AND METHODS

Experimental fish and their maintenance

A total number of 90 healthy fishes *Clarias batrachus* collected from local fish market of Meerut district with average length of 15±2 cm and average weight upto 60±8 gm. Collected fishes were immediately washed for 5 minutes in 0.01% KMnO₄ to avoid any dermal infection. After washing fishes were acclimatized for 15 days in laboratory condition using tap water in glass aquarium. Fifty percent of water was changed daily during acclimatization. The water quality of aquarium was monitored (pH- 7.2±0.06, Temperature - 23± 2).

Calculation of LC₅₀ of cadmium sulphate

Acclimatized fishes were exposed to different concentration of Cadmium Sulphate (2,4,6,8,10,12,14 mg/l) for determination of LC₅₀ value. Cumulative mortality will be determined after 96 hours. The dead fishes will be removed once they will be observed. LC₅₀ value will be determined by probit analysis by 96 hours median tolerance limits (Litchfield and Wilcoxon, 1949) LC₅₀ value of Cadmium sulphate for *Clarias batrachus* was 1.39mg/l.

Experimental design

Acclimatized and healthy 90 fishes were taken out for the experiment and divided into three equal groups of 30 fishes. The group I of fishes were used as a control, group II of fishes was treated Cadmium Sulphate with

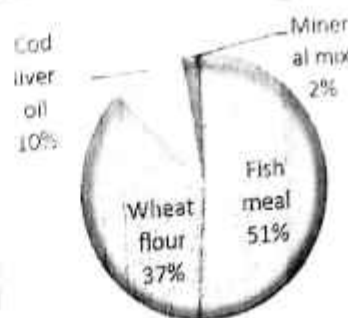


Fig. 1: Pie chart showed the ingredient of Basal diet in percentage

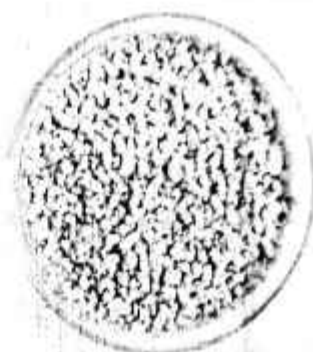


Fig. 2: Basal diet.

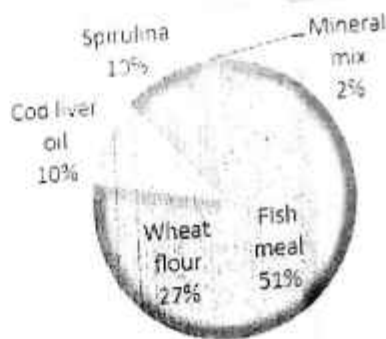


Fig. 3: Pie chart showed the ingredient of *Spirulina platensis* Supplemented Diet diet in percentage

basal diet and the group III of fishes was treated Cadmium Sulphate with *Spirulina platensis* supplemented diet 10% of their body weight. The water was changed every second day and the fishes were fed twice a day. After 30 and 45 days of exposure time the fishes were removed from all the three groups and they were sacrificed by decapitation and kidney was removed for histopathological studies. Histopathological section immediately fixed in 10% formalin solution and

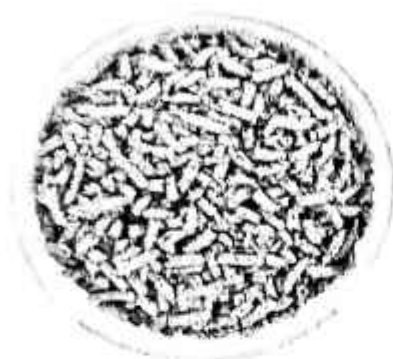


Fig. 4: *Spirulina platensis* supplemented diet.

histopathological slides was prepared. The histopathological of kidney slide was observed under an Olympus research microscope and photographs was taken and analysed to observed the difference among the all three groups.

Preparation of diet

Acclimatized fishes were fed with basal diet (contained 35% protein) 10% of their body weight twice a day (Sharma and Jha, 2010). Basal diet (Fig. 2) was prepared using fish meal (51.25%), wheat flour (36.75%) Cod liver oil (10.00) and minerals (2%). While, supplemented (Fig. 4) diet was prepared replacing same quantity of wheat flour with *Spirulina platensis* as described by James *et al* (2009).

RESULTS AND OBSERVATION

Kidney is the vital organ of excretion and osmoregulation and it also help in monitoring the homeostatis. It also responsible for selective reabsorbtion

which help in maintaining the volume and pH of blood and erythropoiesis (Iqbal *et al*, 2004). In this study, the histopathological slides of kidney of *Clarias batrachus* in group I, T.S. of control fish showed normal architecture without any histopathological alteration with well arranged haemopoietic tissue (Fig. 5). In group II treated with cadmium sulphate, T.S. of kidney of fish *Clarias batrachus* showed negative impact on the kidney after 30 days duration of time (Fig. 6). It showed tubular and haemopoietic tissue vacuolisation with widened Bowman's spaces and deshapecd glomerulus with necrosis of cells. These histopathological alteration depends upon the dose and duration of time. These histopathological lesions in kidney of fish in this group showed more severe alteration after 45 days (Fig. 7), which showed tubules vacuolisation with deshapecd uriniferous tubules, widen Bowman's spaces with deshapecd glomerulus, interstitial necrosis with hypertrophy and aggregation of cells. These result were similar to Dar *et al* (2011) whose finding showed the negative impact in kidney of *Clarias batrachus* after being exposed to 8ppm Cadmium Chloride for 30 and 60 days which exhibits several histopathological alteration loosening of haemopoietic tissue, formation of clusters and lumps in haemopoietic tissue, deshapecd of uriniferous tubules, narrowing of tubular lumen vacuolization and degeneration of the cells of uriniferous tubules, increase the shape of renal corpuscles and shrinkage in glomeruli. to increased dose and time period in *Clarias batrachus*. Kirubagaran and Joy (1988) found similar results in the kidney of *Clarias batrachus* after exposure with mercury hypertrophy of epithelium of renal tubule and

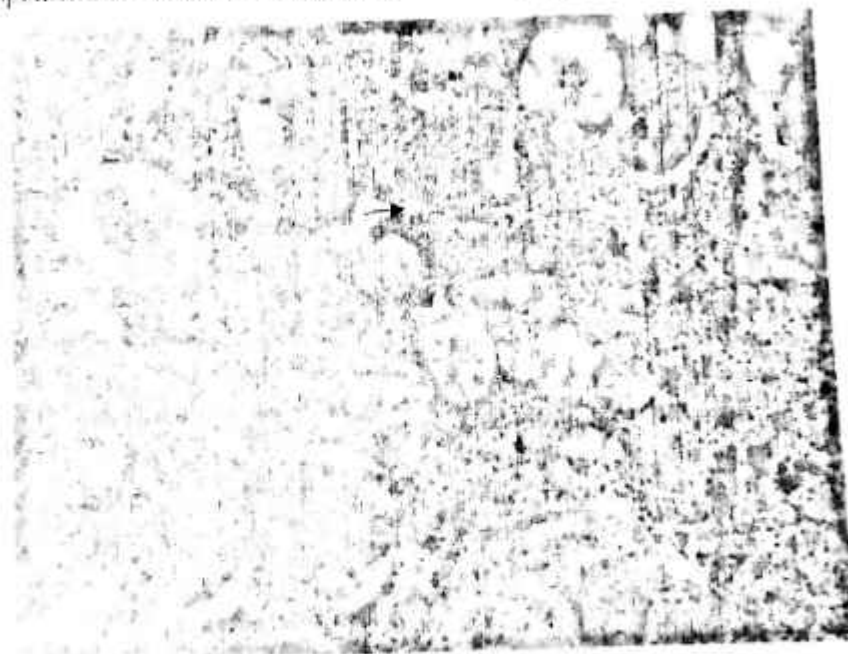


Fig. 5: T.S. of kidneys of *Clarias batrachus* after control period fish showing well arrange haemopoietic tissue. 1—3 uriniferous tubule and well shaped glomerulus with clear Bowman's spaces.



Fig. 6

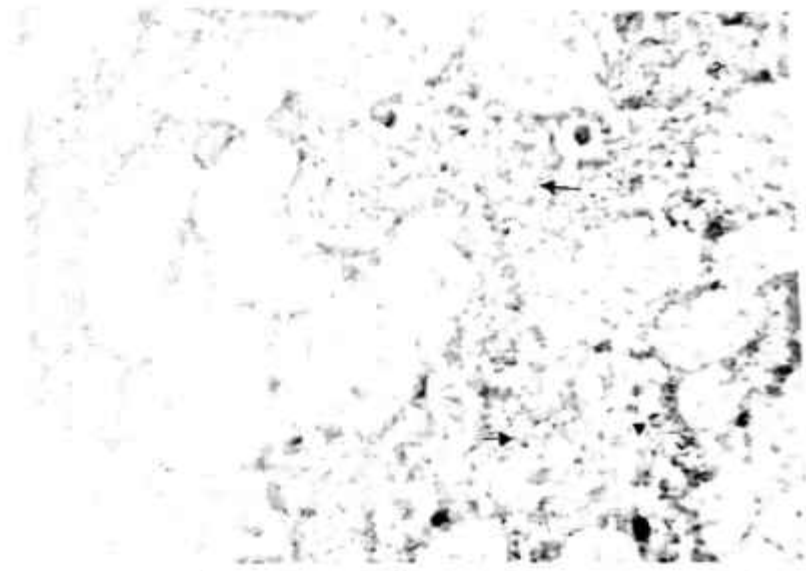


Fig. 7

contraction in Bowman's spaces. Mild edema, reduction in cell size, severe damage and further degeneration of renal tubules, disorganization of renal tissue and necrosis were seen when *T. mossambica* was exposed to sub lethal concentration of Cadmium Sulphate for 20 days under lab conditions (Jalaludeen *et al.* 2012). Similar results were also found by Mohmish and Gaherwal (2020) in *Clarias batrachus* from Arsenic contaminated Chhlpura pond water in Uttar Pradesh, India. Group III treat with *Spirulina platensis* diet along with Cadmium

Sulphate showed some better result in compare to Cadmium Sulphate treated group II and histopathological alteration were less severe in this group. In this group kidney showed less tubular and hematopoietic tissue vacuolization with less amount of de-shaped glomeruli after 30 days (Fig. 8) and after 60 days (Fig. 9), it shows less necrosis and tubular and tissue vacuolisation. Bowman's spaces are less widened with less de-shaped urinitorous tubules as compared to group II respectively. These less curative effect is just because of *Spirulina*

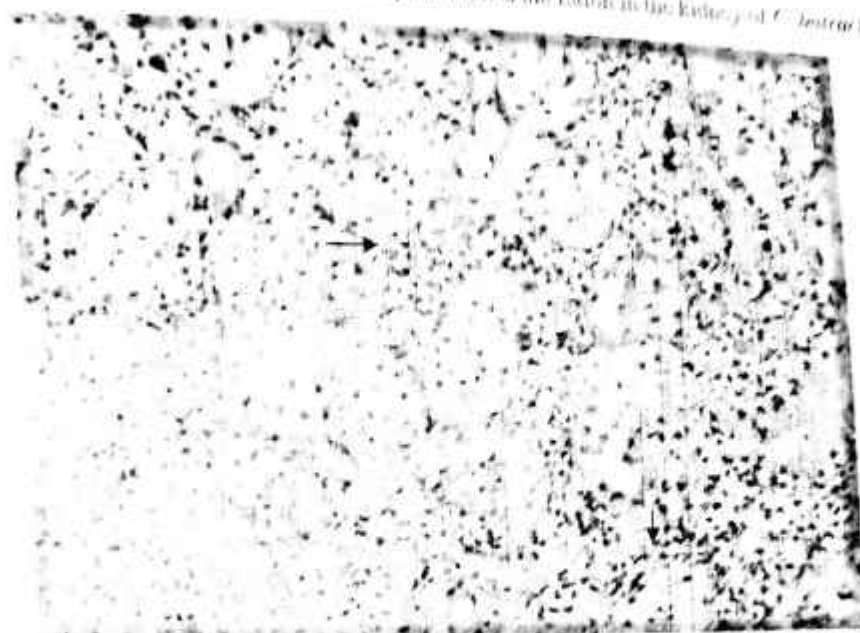


Fig. 8: US (15x) kidney structure of after 30 days fed with *Spirulina* supplemented diet and cadmium sulphate treated fish, *Clarius batrachus* showing less tubular and haemopoietic tissue vacuolisation and (→) with more or less deslumped Glomerulus (↓) with Bowman's space.



Fig. 9: US (15x) kidney structure of fish after 45 days fed with *Spirulina* supplemented diet and cadmium sulphate treated fish, *Clarius batrachus* showing less uriniferous tubule and haemopoietic tissue vacuolisation (↓), less tissue necrosis and compactness of connective tissue (←) with less deslumped glomerulus (→) and hypertrophy of tissue.

platensis supplemented diet which played important role to chelate or detoxify the organism against heavy metal. Dar and Jha (2013) found similar result and observed the protective nature of *Spirulina platensis* against Cadmium Chloride in the kidney of *Clarius batrachus* with different concentration of Cadmium exposure i.e. 4mg/l and 8mg/l after 30 and 60 days duration of time, there result also showed that the 10% *Spirulina platensis* supplemented diet decrease the loosening of

haemopoietic tissue, clustering of cells, necrosis of uriniferous tubules and expansion of Bowman's spaces. Uriniferous tubules and glomerulus are more or less regular shape. These characteristics of *Spirulina* is due to the presence of various antioxidants such as Vitamin B1 and B2, selenium, carotenoids, gamma-linolenic acids and phycoeyanin (Poinero *et al.*, 2001). Due to the presence of these strong antioxidants it has free radical scavenging properties in addition to its strong chelating

effect (Chen and Pen, 2005). *Spirulina platensis* also improves the histopathological alteration in kidney of rats affected by the toxicity of Cisplatin by Kuhad *et al* (2006). In this study, *Spirulina platensis* shows its protective behaviour in group III Cadmium affected fish and reduced histopathological lesions as tubular and haemopoietic tissue vacuolization, necrosis with less deshapaped or contracted glomerulus as compared to group II Cadmium treated fishes.

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Sorghum⁴

Abstract

Intelligent In present, studies were taken different sorghum genotypes including CSV-20, CSV-23, PC-615, CSH-16, CSH-30 and Maldandi. They were screened for their relative susceptibility against the *sitophilus oryzae* under storage. The percentage of seed damage was recorded from 30 DAS up to 180 DAS. This damage ranged from 3.11 (CSH-30) to 51.80 (Maldandi) and seed weight loss percentage ranged from 5.66% (CSH-30) to 36.75% (Maldandi) all among the six different genotypes variety Maldandi was found most susceptible and hybrid CSH-30 was least susceptible comparatively to other genotypes.

Introduction

Sorghum is the most important feed and fodder crop. It was most commonly used in the production of ethanol before recent times (Anonymous 2021). It is also called a camel crop due to its ability to withstand drought and hard conditions. It plays a significant role in food security and millions of poor people in some marginal agricultural areas. It has ranked fifth after wheat, maize, rice, barley, and corn. According to 2018 data total cultivated land in India is 4.96 million hectares while the production of sorghum in India is 4.95 million with 998 kg/ha productivity. Highest production of sorghum area (2.17 mha) in Maharashtra (181/m tones) while productivity is (833 kg/ha) followed by Karnataka area (1.09 mha) with (1.13 tonnes) and productivity (1040 kg/ha) (Agriculture statistics at a Glance 2018).

The kernel damage caused qualitative and quantitative loss and a decline in the percentage of

germination Devietal (2014). In different types of stored cereals *Sitophilus oryzae*, *rice weevil*, and *Linnaeus* (*Coleoptera Curculionidae*) are major pests of the grains. Control of this grain pest by using many synthetic pesticides available on market. It is a common practice for farmers and stockholders. While chemical pesticides have so many side effects like environmental pollution, and health hazards, it causes residual toxicity and their accumulation) in the ecosystem of wildlife including human beings, and develops biological magnification (Metcalf 1975). Recently it has been reported that the genotype of sorghum up-rises the suffer by heavy damage due to rice weevil under storage conditions. Hybrid/variety CHS-BR, CSH-1, and CSH-5, varieties 108 and 370 were screened by Borikr and Taydae in 1979, who reported that these varieties were less susceptible to *Cytophilus oryzae*, compared to the local varieties (R 10 and 604). Kudachi and Balikai 2014 was reported to have heavy contamination of rice weevil in sorghum grain

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damage up to 83.5% was recorded over six months. The best indicator of economic loss of the seed in storage is the percentage of seed weight loss which is caused by rice weevils as reported by Teshome et al (1999).

Venkatrao et al (1958) were to be observed rice weevil infestation alone causing 61.3% sorghum grain losses over a time of 5 months. The data of present studies indicated the importance of rice weevil in stored sorghum hence, a trial was conducted on newly released varieties/hybrids to know their reaction.

Material Method

In present studies have taken different sorghum hybrids/varieties CSV-20, CSV-23 and PC-b15, CSH-16, and CSH-30 (Maldandi) to know the reaction of *S. oryzae* rice weevil in storage. All the healthy unaffected types of each genotype of grain were dried in hot air for six hours at 42 degrees Celsius for removal of infestation due to stored grain pests. During the experimentation weight of every variety (100 gm) was taken with 10.0±2 moisture content and then kept in 500 ml capacity of plastic bottles. The top of every bottle was covered with muslin cloth by introducing 10 pairs of five days old weevils. Bottles were tightly fixed by a rubber band with four replications and were taken into observation for up to 180 days. During the investigation damaged percentage of seed and

percentage of weight loss were recorded during the above period.

All the percentage of weight loss was worked out by using the formulas given by Adams (1976) and Adam Schulten (1978). Collective data were subjected to statistical analysis.

$$\text{Percent of weight loss} = \frac{UND - DNU}{U(ND + NU)} \times 100$$

U=weight of uninfested grain (g)

NU= Number of uninfested grain

D= weight of infested grain

NU= Number of infested grains

Result and discussion:- In Table 1 results are given that show the average seed damage in different genotypes of sorghum in 30 days of storage (DAS) CSH-30 ranged from 1.04 to 31.50 on 60 days (DAS) ranged from 1.56 to 36.50 and on 90 days it varies 2.35 to 50.60 and on 120 days (DAS) 3.65 to 57.80 and 4.72 to 63.40 on 15 days after storage and 5.35 to 71.00 on 180 (DAS). The overall seed damage percent ranged from 3.11 percent to 51.80 percent, hybrid CSH-30 recorded minimum seed damage of 3.11% as compared to all other varieties and hybrids. The local check Maldandi recorded maximum damage of 51.80% to the variety CSV-23 and hybrid CSH-16 were other genotypes that showed the least damage.

Table-1 Reaction of Different genotypes to *sitophilus oryzae*

Genotype	Seed damage % Days after storage						Mean
	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	
CSV20	3.50	7.50	15.50	24.00	31.00	37.50	19.83
CSV23	1.50	2.50	9.00	16.50	21.00	24.00	12.42
PCG15	3.50	7.00	16.50	25.50	32.00	43.50	21.33
CSH16	1.50	6.00	12.50	19.00	22.50	27.00	14.75
CSH30	1.04	1.50	2.35	3.65	4.72	5.35	3.11
Maldandi local check	31.50	36.50	50.60	57.80	63.40	71.00	51.80
Sem I CD at 5%	0.14	0.33	0.23	0.31	0.58	0.92	

Kudachi and Balitai 2014 observed that 83.5 percent over six months, rice weevil alone caused damage.

Table 2 shows that grain weight loss percentage varies from 1.96 to 16.50 on 30 DAS, 2.69 to 24.00 on 60 days after storage on 90 days it varies from 3.60 to 29.00 and from 6.310 to 41.50 on 120 DAS on 150 days it varies from 8.43 to 52.50 and 10.95 to 57.00 on 180 days after storage. By consideration, the minimum seed weight loss was recorded in CSH-30 (5.66%) and the maximum in

Maldandi at 36.15. The damage in other entries ranged from 13.08 % (CSH-16) to 19.75% (PC-b15) CSV-23 was close to CSH 60 in recording the seed weight loss. Results show that sorghum varieties suffered significant seed damage under the storage however other hybrids suffered less damage by rice weevil compared to other varieties.

Table 2. The reaction of genotypes to *sitophilus oryzae* (grain weight loss percentage)

Genotype	Seed damage % Days after storage						
	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS	Mean
CSV-20	0.50	2.50	11.00	19.00	29.00	34.00	16.08
CSV-23	6.63	7.79	9.80	15.28	20.95	28.96	14.90
PC6-15	5.50	11.00	16.50	24.00	29.00	32.50	19.75
CSH-16	5.68	7.73	8.40	14.40	18.46	23.84	13.08
CSH-30	1.96	2.69	3.60	6.310	8.43	10.95	5.66
Maldandi local check	16.50	24.00	29.00	41.50	52.50	57.00	36.75
Sem I CD at 5%	0.31	0.28	0.32	0.33	0.42	0.58	

These results were similar to the finding of Kodachi and Balikai 2014 reporting that genotype IS 2312 shows a high degree of resistance by a recording of minimum wt. loss of 0.23% under storage followed by IS 2205 (0.38%) after one month of storage whereas the percentage of grain damage in different varieties varies from CSH-5 (6.50 to 21.17) CSV-4 under the storage of 45 days was observed by Kishore et al (1975). Various resistance of sorghum genotype to rice weevil was studied by Krishan Murthy et al (1976) during this study it was reported that variety CSH-5 was fairly resistant to pests while M-35-1 was highly infested by the storage pest rice weevil. It was reported by Borikar and Tayade, that hybrids were less affected by rice weevil compared to the local genotypes due to the genotypic character of the thick pericarp. According to him, CSH-5 was fairly resistant to pests and M.35-1 was infested by a large number of rice weevils. Borikar and Tayade (1979) observed that hybrids comparatively had a loss incidence of rice weevil than local genotypes. This is due to having a genotypic character with a thick pericarp.

Conclusion

During the storage of cereals like wheat, maize, rice, and sorghum insect pests are the major pest problem and future production of sorghum. The use of resistant varieties/hybrids is the best strategy to avoid the above problem. Because of these economically safe, alternatives of reducing the loss of grains after post-harvest due to insect pest rice weevil causes high loss in grain weight and term of quality.

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Efficacy of some botanical powders against rice weevil, *Sitophilus oryzae* infesting sorghum seed under storage conditions

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ABSTRACT

The present studies were undertaken to evaluate the efficacy of some botanical powders against rice weevil infesting stored sorghum seed. In all twelve treatments and one untreated control were used. The bark powder of *C. zeylanicum* was found to be the most effective treatment amongst all the botanical powders, recording 100% adult mortality, while *A. calamus* used as standard check recorded 92.25% mortality. The seed powder of *A. squamosa* (92.74%) and *A. Indica* (85.33%) also gave better protection. Data recorded on percent weightloss was also minimum in *C. zeylanicum* (1.45%) followed by *A. Squamosa* (1.74%) and *A. calamus* (1.78%) while untreated control showed 44.33% weight loss.

Key words : *A. calamus*, *A. Indica*, *A. Squamosa*, botanical powder, *C. Zeylanicum*, *Sitophilus oryzae*, sorghum.

INTRODUCTION

Sorghum, which is a most important feed and fodder crop, is being exploited for alternate uses especially to produce ethanol in recent times (Anonymous 2021). It is now ranked fifth after wheat, maize, rice and barely and possess hardness, high tolerance to drought. The rice weevil, *Sitophilus oryzae* is highly damage causing pest of raw stored cereals in the world. The rice weevil is known to cause 18.30% losses in storage (Shashi Bala, 2020). The kernel damage caused by *S. oryzae* attracts other pest species. Its damage cause qualitative loss and decline in the germination (Devi *et al.*, 2014).

There is no doubt that chemicals have played a significant role in the past in protection of seed in storage. However, their excessive and inappropriate use in our agro-ecosystem in the last few decades or so has resulted in degradation of our environment. Surveys in the country have indicated that food commodities are highly contaminated with persistent pesticides residues. Botanical pesticides are generally pest-specific and have several advantages in relation to synthetic compounds (Mishra and Bala, 2019; Suleiman and Rugumamu, 2017). They can be new classes of safer insect control agents and can be produced by farmers and small industries. Present studies

were undertaken to evaluate different plant powders for their efficacy against *S. oryzae*.

MATERIALS AND METHODS

Present experiment was carried out under laboratory conditions at Department of Zoology, Meerut College, Meerut. Rice weevil, *Sitophilus oryzae* culture was initiated by collecting the adults from the infested sorghum grain samples maintained in the Department. Newly emerged adults of same generation were collected from culture initiated for present studies. The insects emerged after four weeks were removed for carrying out this experiment. Seven to fourteen day old weevil adults were sexed and used in the experiment.

Plant materials used : Plant materials that were exploited for their potentiality as natural seed protectant are : Shivari (*Vitex negundo* L.), Kranj (*Pongamia glabra* L.), Sweet flag (*Acorus calamus* L.), Tulsi (*Oscimum basilicum* L.), Custard apple (*Annona squamosa* L.), Malabar nut, (*Adathoda vesica* L.) Neem seeds and leaves (*Azadirachta indica* A. juss), Pwriwinkle (*Vinca rosea* L.) Turmeric (*Curcuma longa* L.), Cinnamon bark (*Cinnamomum zeylanicum* L.) and Coriander seeds (*Coriandrum sativum* L.). There were total twelve treatments plus one untreated check (Tables 1-2). Amongst the treatments *Acoruscalamus* was included as

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Table 1. Adult mortality (%) of *Sitophilus oryzae* as influenced by different botanicals

Treatment	Part	Dosage (%) (w/w)	Adult mortality (%) of <i>Sitophilus oryzae</i> at days after storage									Mean
			7	14	21	30	60	90	120	150	180	
<i>Coriandrum sativum</i>	Seeds	5	38.00 (38.05)	41.67 (40.20)	41.67 (40.20)	43.67 (41.36)	39.00 (39.05)	32.67 (34.84)	26.33 (30.86)	22.33 (28.18)	19.33 (26.00)	33.85
<i>Pongamia glabra</i> L.	Fruits	5	61.33 (51.54)	69.33 (56.37)	70.33 (56.99)	78.00 (62.02)	74.33 (59.67)	64.33 (53.32)	57.33 (49.21)	46.67 (43.08)	47.67 (43.66)	63.27
<i>Pongamia glabra</i> L.	Leaves	5	30.67 (33.60)	36.67 (37.25)	42.33 (40.58)	50.67 (45.38)	48.67 (43.85)	41.33 (40.00)	35.67 (36.66)	32.67 (34.84)	29.33 (32.79)	38.67
<i>Acorus calamus</i> L.	Rhizome	1	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00
<i>Oscimum basilicum</i> L.	Leaves	5	27.67 (31.72)	37.67 (37.85)	42.00 (40.39)	39.33 (38.83)	38.33 (39.02)	29.33 (32.79)	24.33 (29.55)	23.67 (29.10)	28.00 (31.93)	32.26
<i>Annatis squamosa</i> L.	Seeds	5	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	95.33 (77.57)	95.00 (77.11)	87.00 (68.87)	97.46
<i>Adathoda vesica</i> L.	Leaves	5	39.33 (38.83)	43.00 (40.97)	57.33 (49.22)	48.00 (43.85)	49.33 (44.61)	41.00 (39.81)	34.00 (35.66)	28.00 (31.93)	24.33 (29.55)	40.48
<i>Azadirachta indica</i> A. Juss	Seeds	5	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	96.00 (78.51)	96.33 (79.13)	88.33 (70.05)	97.85
<i>Azadirachta indica</i> A. Juss	Leaves	5	60.67 (51.15)	69.67 (56.58)	75.33 (60.23)	76.00 (60.67)	73.33 (58.98)	61.33 (51.54)	57.00 (49.02)	46.67 (43.08)	42.00 (40.39)	62.44
<i>Vinca rosea</i> L.	Leaves	5	17.33 (24.25)	23.33 (26.88)	24.67 (29.77)	24.00 (29.33)	24.33 (29.12)	20.67 (27.03)	18.00 (25.10)	15.00 (22.78)	13.33 (21.40)	20.07
<i>Curcuma longa</i> L.	Rhizome	5	26.33 (30.86)	32.67 (34.84)	34.00 (35.86)	34.33 (35.86)	29.33 (32.89)	22.33 (28.18)	18.67 (25.59)	15.67 (23.28)	13.00 (21.10)	25.15
<i>Cinnamomum zeylanicum</i>	Bark	5	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	100.00
<i>Vitex negundo</i> L.	Leaves	5	53.3 (46.9)	56.0 (48.44)	61.3 (51.54)	62.6 (52.33)	61.3 (51.54)	82.00 (64.91)	51.3 (45.76)	51.33 (45.76)	48.0 (43.85)	58.59
Untreated check	-	-	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
SEM ±			0.51	0.60	0.55	0.31	0.59	0.50	1.22	0.78	0.49	0.22
CD at 5%			1.98	2.33	2.15	1.19	2.30	1.94	4.74	3.05	1.90	0.86

*Figures in the parentheses are arcsine transformed values.

standard check to assess other treatments for their efficacy against *S. oryzae*. All the treatments were assessed for adult mortality (%) and seed weight loss (%) and The data for each parameter were recorded at monthly intervals (Tables 1-2). All the treatments were applied @ 5% powder except *Acorus calamus* rhizome powder which was applied at the rate of 1%.

Preparation of different plant materials and sorghum seed : Various plant materials collected from different sources were turned into powders and used in this study. Each powder was first coated over sorghum seeds then was tested against

adults of *S. oryzae* (Shashi Bala, 2020). The seeds of local variety weighing 100 g were put in 250 g plastic container for each plant material and mixed thoroughly in each container at the rate of 5% (w/w), while control treatment was run without any treatment (with no material added). Four such sets were maintained for each plant material. The experiment was conducted under complete randomized block design, replicated four times. The mixed contents in each plastic container were allowed to rest for about 30 minutes before introducing five day old 10 pairs of adult weevils into each plastic container. Perforated muslin cloth

Table 2. Seed weight loss (%) due to *Sitophilus oryzae* as influenced by different botanicals.

Treatment	Part	Dosage (%) (w/w)	Loss in grain weight (%) days after storage						Mean
			30	60	90	120	150	180	
<i>Coriandrum sativum</i>	Bark	5	4.63 (12.43)	5.39 (13.42)	7.30 (15.68)	8.34 (16.81)	11.92 (20.19)	15.48 (23.17)	8.84
<i>Pongamia glabra</i> L.	Fruits	5	2.58 (9.23)	4.26 (11.91)	5.40 (13.44)	7.52 (15.92)	9.40 (17.85)	12.36 (20.58)	6.92
<i>Pongamia grabra</i> L.	Leaves	5	3.27 (10.42)	5.43 (13.47)	8.32 (16.76)	9.53 (17.98)	12.35 (20.57)	15.76 (23.38)	9.11
<i>Acorus calamus</i> L.	Rhizome	1	0.00 (0.00)	0.00a (0.00)	0.00 (0.00)	0.00 (0.00)	6.28 (14.51)	4.41 (12.12)	1.78
<i>Oscimum basilicum</i> L.	Leaves	5	6.24 (14.47)	7.81 (16.23)	9.83 (18.27)	12.27 (20.50)	18.46 (25.44)	20.34 (26.80)	12.49
<i>Annona squamosa</i> L.	Seeds	5	0.00 (0.00)	0.00 (0.00)	0.84 (5.25)	1.26 (6.45)	1.57 (7.20)	2.36 (8.84)	1.01
<i>Adathoda vesica</i> L.	Leaves	5	5.29 (13.25)	6.38 (14.63)	7.34 (15.71)	10.32 (18.74)	15.53 (23.21)	18.34 (25.36)	10.53
<i>Azadirachta indica</i> A. Juss	Seeds	5	0.00 (0.00)	0.00 (0.00)	1.32 (6.59)	1.54 (7.13)	1.86 (7.83)	3.19 (10.27)	1.32
<i>Azadirachta indica</i> A. Juss	Leaves	5	1.55 (7.15)	2.63 (9.33)	4.37 (12.06)	7.27 (15.64)	12.46 (20.67)	15.39 (23.10)	7.28
<i>Vinca rosea</i> L.	Leaves	5	8.37 (16.81)	10.42 (18.83)	12.56 (20.75)	15.78 (23.40)	22.21 (28.12)	25.77 (30.47)	15.84
<i>Curcuma longa</i> L.	Rhizome	5	7.38 (15.76)	8.58 (17.03)	10.49 (18.90)	12.47 (20.68)	18.27 (25.30)	20.68 (27.05)	12.98
<i>Cinnamomum zeylanicum</i>	Bark	5	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.43 (0.00)	3.27 (0.00)	1.45
<i>Vitex negundo</i>	Leaves	5	2.03 (18.19)	3.04 (10.80)	4.41 (12.12)	6.28 (14.51)	8.28 (16.72)	11.30 (19.64)	5.85
Untreated check			23.67 (29.11)	24.63 (29.75)	28.55 (32.30)	34.70 (36.08)	62.76 (52.38)	73.74 (59.14)	41.34
SEm ±			0.10	0.10	0.08	0.09	0.08	0.15	0.05
CD at 5%			0.38	0.40	0.32	0.34	0.31	0.59	0.20

Figures in the parentheses are arcsine transformed values.

was used to cover the opening of each container to ensure good aeration. The containers were placed in an incubator maintained at a temperature of $28 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH. The content of each of the plastic container was poured in a dish and dead or alive adults were counted.

Adult mortality counts : Mortality count in each treatment was recorded at 7, 14, 21, 30, 60, 90, 120, 150 and 180 days after storage. The insects were allowed to mate and oviposit for 14 days. All adults in both treated and untreated bottles were removed after 14 days and each bottle was monitored up to 180 days. At the end of the period, the number of emerged adults was counted.

Statistical analysis : Corrected mortalities were estimated by using Abbott's formula (Abbott, 1925).

Estimation of seed weight loss : Observation on percentage weight loss were recorded by counting number of uninfested seeds and number of infested seed weight loss were worked out by using formula (Adam and schulton, 1978)

$$\text{Percentage weight loss} = \frac{(UND) - (DNU)}{U(ND + NU)} \times 100$$

where, U = Weight of uninfested seeds, NU = Number of uninfested seeds, D = weight of infested seed, ND = number of infested seeds.

RESULTS AND DISCUSSION

The overall means on seed damage in all botanicals indicated that they significantly gave better protection against seed damage as compared to untreated check (46.92%). The minimum seed damage was recorded in bark of *C. zeylanicum* (1.55%) followed by seed powder of *A. squamosa* (4.33%) and rhizome powder of *A. calamus* (4.95%). All the other botanicals were significantly better than untreated check (46.92%) where the seed damage percentage ranged from 13.02% (*C. sativum*) to 43.89% (*V. rosea*).

On 30th day after storage four botanicals viz., *A. calamus* rhizome powder *A. squamosa* seed powder, *A. indica* seed powder and *C. zeylanicum* bark powder recorded no seed weight loss as compared to untreated check which recorded 23.67% seed weight loss. In general, all the botanicals recorded less than 10% seed weight loss as compared to untreated control. On 60th day after storage again four botanicals *A. calamus* rhizome powder *A. squamosa* seed powder *A. indica* powder and *C.*

zeylanicum bark powder showed no seed weight loss as compared to untreated check (24.63%). All other botanicals showed variable efficacy in reducing seed weight loss. On 90th days after storage the seed treated with *C. zeylanicum* bark powder and *A. calamus* rhizome powder showed no seed weight loss while maximum seed weight loss was recorded in check (28.55%). The seed powder of *A. squamosa* and *A. indica* were equally effective in reducing the seed weight loss. On 120th days after storage bark powder of *C. zeylanicum* recorded no seed weight loss as compared to untreated check (58.70%). The seed powders of *A. squamosa* and *A. indica* were significantly better than all the other botanicals in reducing seed weight loss. There was gradual decline in the efficacy of different botanicals. On 150th day after *A. squamosa* recorded minimum seed weight loss (1.57%) followed by *C. zeylanicum* (5.43%) and *A. calamus* (6.28%) and seed powder of *A. indica* rest of the botanicals were significantly better in reducing seed weight loss than untreated check. The maximum seed weight loss was observed in untreated check (62.76%). On 180th days after storage the minimum seed weight loss was recorded in *A. squamosa* (2.36%) followed by *C. zeylanicum* (3.27%) and *A. calamus* (4.41%). The untreated check recorded very high seed weight loss (67.67%). All the other botanicals were not very effective in bringing down seed weight loss.

Adult mortality (%) days after storage : The population build up in various treatments was variable as expressed in the mortality of the adults (Table 1). The data on adult mortality were recorded from 7th day of storage after treatment and release of adults in each treatment. The data were further recorded on 14, 21, 30, 60, 90, 120, 150 and 180 days after storage in all the treatments. On 7th days of treatment the maximum mortality was observed in rhizome powder of *A. calamus*, seed powder of *A. squamosa*, seed powder of *A. indica* and bark powder of *C. zeylanicum* (100%) while there was no mortality in untreated check. In all the other treatments the adult mortality ranged from 24.67% (*O. basilicum* leaves powder) to 61.33% in (*A. indica* leaves powder). On 180th day after storage the bark powder of *C. zeylanicum* gave 100% adult mortality followed by rhizome powder of *A. calamus* (95.33%) and seed powder of *A. squamosa* (87.0%) while seed powder of *A. indica* recorded 61.33% adult mortality. In rest of the

botanicals adult mortality ranged from 15.00% (*V. rosea*) to 39.33% (*V. negundo* leaves powder). There was no mortality in untreated control. The overall means for each botanical treatment revealed that the bark powder of *C. zeylanicum* gave 100% adult mortality throughout the experimentation schedule. This botanical treatment was closely followed by rhizome powder of *A. calamus* (92.25%) which was used as standard check. The seed powder of *A. squamosa* (92.74%) and seed powder of *A. indica* (88.33%) also provided good adult mortality. Rest of the other plant materials were not much effective.

Seed weight loss (%) at days after storage : The data were recorded on seed weight loss in different botanical treatments at 30, 60, 90, 120, 150 and 180 days after storage (Table 2). Taking the overall means (30, 60, 90, 120, 150 and 180 days after storage) of all the botanicals it can safely be concluded that *C. zeylanicum* bark powder showed minimum seed weight loss (1.45%) followed by *A. squamosa* (1.74%) and *A. calamus* (2.82%) as compared to untreated check (44.33%). The seed powder of *C. sativum* and *A. indica* recorded less than 10% seed weight loss. Rest of the botanicals were also significantly more effective in checking the seed weight loss as compared to untreated check..

Gadewar *et al.* (2017) used of sweet flag powder against *S. oryzae* in sorghum and reported very satisfactory control of the weevil. Jasrotia and Singh (2019) assessed seven different botanicals in wheat against *S. oryzae* and observed that high mean corrected mortality in rhizome powder of *A. calamus* followed by eucalyptus leaf powder. Botanical powders were significantly more effective against *S. oryzae* when compared to untreated control (Govindan and Nelson, 2009). Bark powder of *C. Zeylanicum* was best treatment in controlling the weevils (Sunilkumar, 2003).

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EFFECT OF CASTRATION ON PHOTO PERIODICALLY INDUCED FOOD INTAKE AND FATTENING IN RED HEADED BUNTING (*EMBERIZA BRUNICEPS*)

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ABSTRACT: Castration prior to photo stimulation does not have a marked influence on body fattening and food intake. However, it affects fattening once the stimulated gonads are removed. Food intake remains substantially uninfluenced. Testosterone therapy does not add to body fat or recovers the effects of castration. Instead, a reduced food intake is recorded in TP treated birds. Long term experiments are, however, recommended to ascertain further the nature of relationship among these three cycles and also to understand the role of steroids in seasonal responses of Red headed Buntings.

Key words: Food intake, fattening, photoperiodism, castration.

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INTRODUCTION

Photoperiod is the principle environment cue that induces seasonal fluctuations and, in general, the circadian rhythms mediate these photoperiodic seasonal metabolic and endocrine responses. This is true for many species of migratory birds as well as number of small mammals (Tewary and Dixit, 1986). The fattening accompanied with weight gain in birds is usually concomitant with the reproductive cycle (Chaudhary, 2020). It is, therefore, assumed that gonads play a role in induced of deposition of fat. It is reported that the gonadal hormones are responsible, at least partly, for hyperphagia, fattening and weight gain. Since then, a number of positive and negative results have accumulated (Singh, 1982). However, still the subject remains controversial.

The redheaded bunting (*Emberiza bruniceps*) is one such form for which now sufficient experimental data are available, and therefore, this species was selected for further investigations. It is a passerine migrant. It is a winter visitor to India. It arrives in fall (September/October), over winters, and returns to its breeding grounds in late March and April. Captive individuals exhibit distinct cycles of fattening and body weight and gonadal activity

(Prasad, 1980). Under laboratory conditions, too, gonadal growth and development, and pre migratory fattening and weight gain are induced by long daily photoperiods. Short photoperiods fails to do so. The present study was aimed to determine- is the gonads (gonadal androgens) influence fattening (weight gain) and food intake in males.

MATERIALS AND METHODS

The red headed bunting (*Emberiza bruniceps*) is an emberizid finch (family- Emberizidae; Order: Passeriformes). It's locally known as Gandam (in hindi). It is common and widely distributed in India, but as winter guest. The individual's measures about 17 cm in length. Sexes are separate; male is yellow with black streaks on back and females are dark brown on back and light yellow belly. The study was performed on male of the species.

After 15 days of acclimatization, the stock birds were regrouped and subjected to different experiments as indicated in next section- experimental design and results. Basically, all birds, expecting NDL (normal day length) controls, were subjected to artificial conditions of day and night. The day of the artificial conditions of day and night. The day of the artificial day/night cycle was created by a 40-watt fluorescent tube providing light



approximately at an intensity of 300 lux. The period of light/dark cycle was controlled by an automatic time-switch (VEB Zeitschalt-electronic Fraunsein GDR, 50 h reserve system). No major electricity break down occurred during the experiments. However, a little disturbance here and there were not considered as they did not occur regularly and repeatedly at the same time. If an electricity failure occurred during day hours, birds were put in sunlight.

The food intake was recorded by placing the groups of birds in cages, which were lined up with polythene sheets up to perch level and placed in trays facilitating spillage collection. The subtraction of final weight (after removal of excreta) from initial weight of food given gave the amount of food consumed by buntings in affixed time.

The amount of food intake/bird/day was obtained by dividing the total amount of food consumed by the number of members in a group. This finally yielded the food intake of one individual in a day. Initially, we recorded the food intake for redheaded bunting housed singly as well as in groups and found that there is no difference between the amounts of food consumed when in group and when housed individually. Tewary (1987) also noted the same thing for *Munia malacca*. Therefore, for the sake of convenience, food intake was recorded periodically when birds were in a group. This was also considered in view that bunting fly and invade grains fields in groups only.

The food intake was always recorded for at least three consecutive days at any observation. The food intake was expressed as food intake (g)/bird/day as well as food intake present of body weight per 24 hours, i.e. food intake per hundred grams of body weight. This was calculated as:

$$\text{Food intake} / \text{Body weight} \times 100$$

The change in body weight was taken of the index of fattening, since in passerines it has already been established that the visceral and subcutaneous fat deposit account for nearly the entire increment in body weight induced by photo stimulation (helm *et al.*, 1967). At each observation, including the beginning and the end of experiments, all birds were individually weighted on a top-pan balance providing accuracy of 0.1 gm. Body weight was expressed in grams. It was also expressed as per cent change in body weight in a few experiments.

The data obtained from different experiments were subjected to statistical analysis. Simple t-test were used for test of difference between two means. But when the two means obtained from the same sample as function of the time were tested, we used paired t- test (Fisher, 1963).

It had two experiments: A and B. Both experiments were performed on males only.

Experiment A : It performed for 60 days. Two groups (n=4) acclimated birds were taken out from laboratory stock (maintain in NDL) and weighted; food intake were also recorded. Then in one group only left of the testes was removed (hemi castrated); in other hand both testes were taken out. Castration was performed uni- or bilaterally. At this time bunting had testis of minimal size (testicular volume 130 mm³, CTW 4.3 mg) indicating inactive gonadal state. The groups were marked as group I (hemi castrated) and group II (castrated).

After 48 hours of castration, both groups were put into long daily photoperiod (15 L/9 D) for 60 days. This was to examine if removal of gonads had any effect on fattening and food intake in male Red headed buntings. After 30 days of long day exposure, body weight and food intake of birds were recorded. Thereafter, birds were given alternate injections of testosterone propionate (TP- Parendren CIBA 1ml = 25 mg TP) for 30 days; olive oil was used as vehicle of the hormone. The doses of TP administered were 12.5 µg bird⁻¹ day⁻¹ in 0.1 ml olive oil for group I and 25 µg bird⁻¹ day⁻¹ in 0.1 ml olive oil for group II. After 15 injections (i.e. after another 30 days in 15L/9D), the body weight and the food intake were gain recorded. The TP administration was done to compensate the androgen production in want to testes. In addition, we had data for intact controls, on respective observations. The data were considered for comparison with the data were considered for comparison with the data of group I and II.

The experimental and husbandry conditions were the same as described in materials and methods.

Experiments B : This experiments was designed to test if removal of testes once their growth initiated had effect on photoperiodic fattening and food intake. Two groups of birds were taken out from the NDL stock and their body weight and food intake were recorded. Then they were castrated.

At this time, these birds had slightly stimulated gonads; CTW ranged from 20-50 mg. The testes have contained profile-rated spermatogonial cells and primary spermatocytes as well. The castrated groups after 48 hours were put into 15L/9D for 15 days to examine the effect of removal of initiated gonads on the fattening and the food intake. Thereafter, one group of them was administered 15 alternate dose of 25 µg bird⁻¹ day⁻¹ of TP in 0.1 ml Vehicle (olive oil) and other received same alternate doses of 0.1 ml of vehicle (olive oil) only. After 15 doses i.e. after another 30 day exposure in 15 L/9D,

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birds were reexamine for fattening and food intake.

RESULTS

Experiments A : The data from this experiment have been demonstrated in Table 1A. Hemi castrated birds put into stimulator long days fattened. Increase in body mass was significant ($P < 0.01$) after 30 days exposure of 15L/9D. Injections of testosterone thereafter did not add to eight of birds, but birds maintained their eight closely. In fully castrated group too fattening occurred ($P < 0.05$), but the weight gain recorded on day 30 was relatively lesser. Testosterone administration to the birds of this group too did not change the body weight markedly.

Intact control birds fattened on exposure to 15L/9D ($P < 0.01$ -Day 30) and maintained an increased body weight ($P < 0.02$) until end of the experiments (day 60). The gain in weight of castrated birds on day 30 was significant lesser ($P < 0.05$) than the gain in weight of intact bird on this day (Table 1A). Also we found a wide difference, though statistically insignificant, between the mean gain in weight of hemi castrated and castrated

group on this 30 the day of 15 L/9D (Table 1A). A comparison of the per cent change in body weight of three groups on 60 the day, however did not reveal any significant variance (Table 2A).

Food intake was almost consistent in hemi castrated and castrated birds until day 30. Thereafter, it was found reduced significantly ($P < 0.001$) on day 60 after T P administration in both the groups. On the other hand sham operated intact controls, food intake was found increased significantly ($P < 0.01$) when observed on 60 the day of the experiment (Table 1A). Food intake % of body weight (food intake/100 gm. of body weight) also appeared trend to decline steadily in all three groups; however, declining trend was steeper in hemi castrated and castrated individual (Table 2B).

Experiment B : Both groups of castrated birds did not exhibit notable change in body weight on day 30. Also, testosterone administration to one of the groups thereafter did not induce fattening (Table 1 B, Fig. 1A & B). Thus, castrated birds put into stimulatory 15L/9D with or without T P did not exhibit noticeable (significant)

Table 1A : Changes in body weight (fattening) and food intake of hemi-castrated, castrated and intact male *E. binniceps* photo stimulated on long days photoperiods (15L/9D) and supplement with or without testosterone

Group	Status of animal	Day of observation	Parameter		
			Body weight	Food Intake	Testis volume (mm ³)
Group I	Hemi-castrated + 12.5ug TP/bird / day (after 30 days)	0	22.60 ± 0.57 (4)	4.50 ± 0.26 (4)	0.130 ± 0.00 (4)
		30	28.52 ± 0.97 (4)	4.10 ± 0.07 (4)	46.09 ± 2.4 (4)
		60	28.32 ± 1.42 (4)	2.60 ± 0.12 (4)	41.54 ± 3.7 (4)
Group II	Castrated + 25 ug TP/bird / day (after 30 days)	0	25.80 ± 0.81 (5)	4.60 ± 0.06 (5)	0.130 ± 0.00 (4)
		30	29.10 ± 0.78 (5)	4.40 ± 0.09 (5)	-
		60	29.40 ± 0.99 (5)	2.60 ± 0.15 (5)	-
Group III	Sham operated (intact)	0	23.50 ± 1.26 (5)	4.40 ± 0.057 (5)	0.130 ± 0.00 (5)
		30	31.40 ± 1.31 (5)	5.34 ± 0.23 (5)	46.42 ± 14.0 (5)
		60	28.80 ± 0.49 (4)	3.63 ± 0.16 (4)	45.23 ± 16.5 (4)

Values are mean ± SE. Figure in parenthesis is for number of individual. Level of significance indicated is with corresponding initial mean. P values: **** < 0.0005, *** < 0.001, ** < 0.01, * < 0.05.

Table 1 B : Fattening and food intake in castrated male *E. binniceps* maintained on 15L/9D and administered with testosterone (TP).

Group	Status of animal	Day of observation	Parameter	
			Body weight	Food Intake
Group I	Castrated + 25 ug (after 45 days)	0	21.47 ± 0.70 (4)	3.60 ± 0.42 (4)
		15	23.40 ± 1.60 (4)	2.90 ± 0.15 (4)
		45	23.05 ± 0.81 (4)	3.46 ± 0.15 (4)
Group II	Castrated + 0.1 ml olive oil/bird / day (after 15 days)	0	21.50 ± 0.82 (4)	2.60 ± 0.37 (4)
		15	22.85 ± 1.62 (4)	3.00 ± 0.10 (4)
		45	24.07 ± 1.42 (4)	3.20 ± 0.26 (4)

Values are mean ± SE. Figure in parenthesis indicates number of individuals.

(P) Prasadham

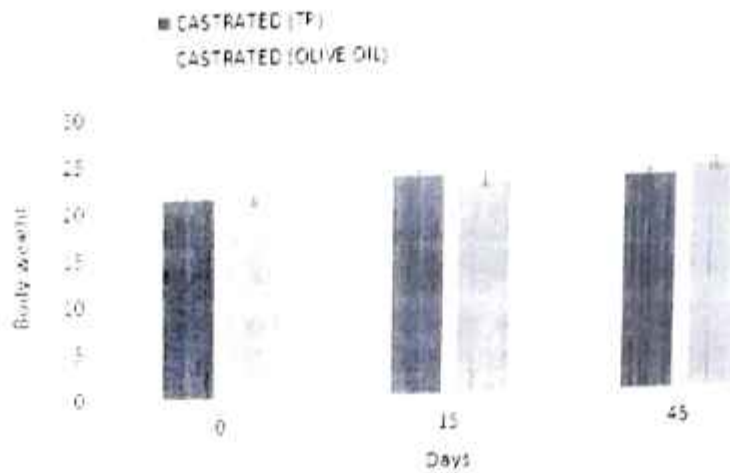


Fig. 1 A: Effect of castration, photoperiod and testosterone therapy on body weight in photo sensitive stimulated male (*Emberiza bruniceps*)

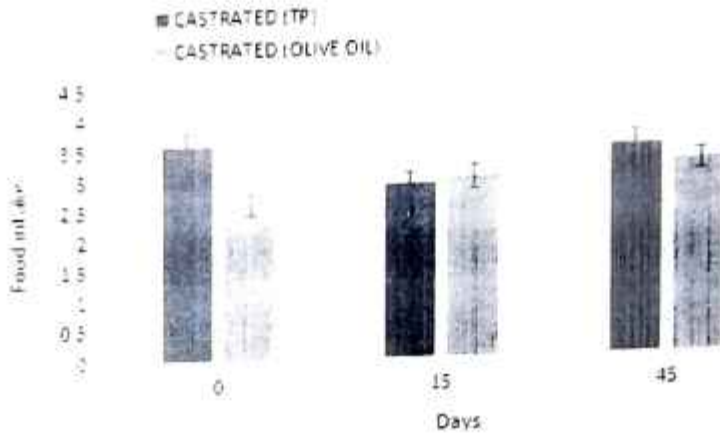


Fig. 1 B: Effect of castration, photoperiod and testosterone therapy on food intake in photo sensitive stimulated male (*Emberiza bruniceps*)

Table 2A: Percent change in body weight (change per 100 gm body weight) of hemi castrated, castrated and intact male *E. bruniceps* held under long day (15L/9D) and administered with or without testosterone.

Group	Status of animal	Observation day	
		(day 30/15)	(day 60/45)
I	Hemi castrated + 12.5 ug TP/bird/day ¹	26.93 ± 5.19 (4)	25.87 ± 9.20 (4)
II	Castrated + 25 ug TP/bird/day ¹	12.66 ± 3.90 (5)	14.27 ± 4.02 (5)
III	Sham operated (Intact)	34.29 ± 6.70 (5)	18.36 ± 8.10 (4)
I	Castrated + 25 ug TP/bird-1 day-1	11.67 ± 5.51 (4)	10.57 ± 4.50 (4)
II	Castrated + 0.1 ml olive oil/bird/day ¹	10.92 ± 4.40 (4)	15.07 ± 3.60 (4)

¹ - units of (ug) 15 and day 45 as the date of two observations
² - 0.05% IAN III of experiment A

fattening, once they had slightly stimulated before exposure to the long days. However, birds maintained good health and a slight elevated but per cent change in body weight did not vary widely from one another (Table 2A)

Food intake remained almost consistent throughout the experiment (Table 1 B, Fig. 1 A & B). Food intake in terms of body weight, i.e. food intake/100 gm. of body weight, also found not to exhibit any trend (Table 2B). Thus, it appears that TP administration to castrated birds,

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Table 2B: Food intake percent of body weight (food intake per 100 gm body weight) of male *E. bruniceps* held under long day (15L/9D) and administered with or without testosterone

Group	Status of animal	Observation day		
		I (day 0)	II (day 30/15)	III (60/45)
I	Hemi castrated + 12.5 ug TP/bird ¹ day ¹	19.7 (4)	15.7 (4)	9.7 (4)
II	Castrated + 25 ug TP/bird ¹ day ¹	17.8 (5)	15.1 (5)	8.8 (4)
III	Sham operated (Intact)	18.7 (5)	17.0 (5)	12.6 (4)
I	Castrated + 25 ug TP/bird-1 day-1	16.7 (4)	12.3 (4)	15.01 (4)
II	Castrated + 0.1 mg olive oil/bird ¹ day ¹	12.1 (4)	13.1 (4)	13.3 (4)

¹Indicate day 15 and day 45 as the date of two observations.

does not have it any effect on either fattening or food intake.

DISCUSSION

In red head buntings, fattening accompanied with weight gain comes concomitantly with the gonadal growth and development. Also, food intake seem to follow a definite trend. This indicates that the gonadal steroids may possibly be involved in regulating food intake, fat deposition and consequent weight gain. An experiment was designed to test this assumption. This experiment consisted of two sub-experiments-exp 2A and exp 2B. Both experiments were performed only on males.

The data from the experiments 2A, which employed hemi castrated and castrated males, suggest that gonadal steroids are not essentially involved in photo-stimulation of fattening in photosensitive Red headed buntings, but that they (androgens) influence the magnitude of fattening in photo stimulated individuals. As evident from the Tables 1A, 2A, both hemi castrates and castrates fattened ($P < 0.01$ and $P < 0.05$, respectively) on 30 the day of their long day photo stimulations, but fat stores were relatively less than the photo stimulated intact. The data on the per cent change in body weight presented in Table 2A indicates that hemi castrated birds deposited fat less than the intact birds and castrated birds had still lesser fat than hemi castrated birds. The difference between the per cent change in body weight of these groups among themselves were statistically insignificant, except for a significant difference ($P < 0.05$) between the body weight of the castrated and intact birds (Table 2A).

Food intake was consistent in all the groups and did not differ widely until day 30. In 15L/9D groups, however, food intake was more ($P < 0.05$) at his date (Tables 1A). Food intake in terms of body weight (food intake per cent body weight) when calculated did not show any increase in any of the groups. Comparatively, it was greater in sham operated intact controls (Tables 2B).

The result of other experiment (experiment B) support the above observations (Tables 1 B, 2A, 2B, Figs. 1 A &

B). In addition, they suggest that the removal of the initiated testes affects the photo periodically induced fattening and weight gain (Table 1B, Figs. 1 A & B). In contrast to the castrates in experiment A, the castrated birds in experiment B, which were castrated at the time of the beginning of migratory periods when their testes slightly stimulated (CTW 5-50 mg), fail to fatten on exposure of 30 days to 15L/9D also did not add a detectable gain in weight (Table 1 B, Figs. 1 A & B). Per cent change in body weight also not remarkable (Table 1 A & B). The data thus suggest that androgens though do not play significant role in photo induction of fattening but they contribute somehow in the magnitude of fat deposition in buntings.

The data from *E. bruniceps* are in agreement with those reported on blackheaded buntings, *E. melanocephala* (Tewary and Kumar, 1981). They have found that the gonadal steroids do not involve in initiation of fattening, but they contribute in maintaining the weight gain once induced in long photoperiods. The results are comparable also with those found on some other species. Stetson and Ericson (1972) reported that intact white-crowned sparrows (*Zonotrichia leucophrys gambelli*) weigh greater ($P < 0.05$) between 20th and 30th day of photo-stimulation. White-throated sparrows (*Zonotrichia albicollis*) do not fatten if they are castrated prior, but not later to photo stimulation (Weise, 1967).

Steroidal hormones are supposed to be involved in producing fattening and feeding behaviour in birds (Baum and Meyer, 1960). In Redheaded buntings, however, testosterone propionate (TP) administered does not induce a fattening or feeding response. Instead, a reduction in food intake was observed in hemi castrated and castrated individuals after 15 alternate injections of T.P. It is possible that the dose of TP administered was not at the threshold level to have an effect on the endocrino-physiological process or that the administration of steroids itself exerts an inhibitory effect. Whatever the present study did not alter the body weight of the birds, but altered the food intake in hemi castrates as well as castrates of experiment

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A (Table 1 A). The data demonstrated as per cent change in body weight and food intake per cent of body weight also reveal the same trend (Table 2 B).

The data are comparable to some other reported findings. It has been suggested that testosterone is possibly involved in the development of migratory fattening and behaviour in males as well as in female birds (Donham, 1979). Testosterone has also been found to affect the duration and intensity of migratory restlessness in males of *Zonotrichia* and *Fringilla* (Weise, 1967). Injections of prolactin and testosterone induces fattening, but not migratory restlessness, in *Zonotrichia leucophrys gambelli* (Yokoyama, 1976).

Gonadal hormones have been found to be affect the feeding behaviour and the body weight in several mammals. Low doses of testosterone propionate reverse the inhibitory effects of castration on food intake and weight gain in male rats. But, at the same time, estrogens exert an inhibitory influence on body weight and food intake in females (Wade, 1976).

To sum up, it appears that the body fattening (weight gain-loss) gonadal (gonad development-involution) cycles are interrelated in Red headed bunting. Castration does not have a significant effect in stimulation of fattening in photosensitive unstimulated individuals but does have an effect on the magnitude of fattening. Castration to simulated photosensitive birds causes reduction in weight gain. Food intake, however, remains unaffected by removal of gonads. Treatment with testosterone to hemi castrates and castrates does not seem to reverse the effect of castration. Instead, a reduction in food intake could be obtained. Further long term experiments are therefore necessary to reveal some more interesting feature of the interrelationships among the cycles of fattening, food intake and reproduction and also the interactions of steroidal hormones and photoperiod in regulation of gonad development, body fat and food intake in the migratory Red-Headed bunting (*Emberiza bruniceps*).

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Correlation Analysis of Reactivity in the Oxidation of Some Aliphatic Secondary Alcohols by Imidazolium Dichromate: A Kinetic Study

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Abstract: Oxidation of several aliphatic secondary alcohols by Imidazolium dichromate (IDC) in dimethylsulfoxide (DMSO) leads to the formation of corresponding ketones. The reaction is first order each in IDC. A Michaelis-Menten type of kinetics are observed with respect to the alcohols. The reaction is catalysed by hydrogen ions. The hydrogen-ion dependence has the form: $k_{obs} = a + b[H^+]$. The oxidation of benzhydrol- α -d (PhCDOHPh) exhibited a substantial primary kinetic isotope effect ($k_H/k_D = 5.79$ at 298 K). The oxidation of 2-propanol has been studied in nineteen different organic solvents. The solvent effect has been analysed using Taft's and Swain's multiparametric equations. The reaction was subjected to both polar and steric effects of the substituents. A mechanism involving transfer of hydride ion from alcohol to the oxidant, via a chromate ester, has been proposed.

Keywords: Alcohols, correlation analysis, dichromate, kinetics, oxidation, mechanism

1. Introduction

Selective oxidation of organic compounds under non-aqueous conditions is an important reaction in synthetic organic chemistry. For this a number of different chromium (VI) derivatives have been reported[1-4]. They are insoluble in most of the organic solvents also. To overcome these limitations, a large number of organic derivatives of Cr(VI) have been prepared and used in synthetic organic syntheses as mild and selective oxidants in non-aqueous solvents. One such compound is Imidazolium dichromate (IDC)[5]. It is known, however, that primary and secondary organic compounds sometimes follow different mechanistic pathways. Such examples are well known in many reactions of primary and secondary halides[6]. It is known that the mode of oxidation depends on the nature of the counter-ion attached to the chromium anion. We have been interested in kinetic and mechanistic aspects of oxidation by complexed Cr(VI) species and several reports, by dichromates have already been reported[7-10]. Therefore, in continuation of our earlier work, we report here the kinetics and mechanism of oxidation of nine aliphatic primary alcohols by IDC in dimethylsulphoxide (DMSO) as solvent. The mechanistic aspects are discussed. The main aims of the present investigation are to (i) determine kinetic parameters and to evaluate the rate laws, (ii) to study the correlation analysis of effect of structure on reactivity and (iii) to postulate a suitable mechanism for the oxidation process.

2. Experimental

2.1 Materials and Methods: All the alcohols were commercial products (Fluka) and were dried over anhydrous magnesium sulphate and then fractionated. Benzhydrol was recrystallised from ethanol. IDC was prepared by the reported method[5] and its purity was checked by an iodometric method. Benzhydrol- α -d (PhCDOHPh) was also prepared by reported method[11]. Its isotopic purity was, as ascertained by its n.m.r. spectra, was $96 \pm 5\%$. Due to the non-aqueous nature of the solvents, p-toluenesulphonic acid (TsOH) was used as a source of hydrogen ions. Solvents were purified by their usual methods[12].

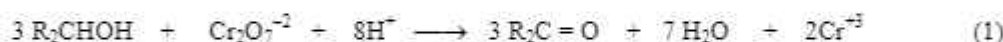
2.2 Product Analysis: The product analysis was carried out under kinetic conditions, i.e. with an excess of the alcohol over

IDC. In a typical experiment, 2-propanol (0.05 mol) and IDC (3.54 g, 0.01 mol) were made up to 100 ml in DMSO and the reaction mixture was kept in dark for ≈ 10 h to ensure completion of the reaction. The solution was then treated with excess (200 ml) of a saturated solution of 2,4-dinitrophenylhydrazine in 2 mol dm^{-3} HCl and kept overnight in a refrigerator. The precipitated 2,4-dinitrophenylhydrazone (DNP) was filtered off, dried, weighed, recrystallized from ethanol, and weighed again. The yields of DNP before and after recrystallization were 2.52 g (88%) and 2.04 g (86%) respectively, based on the consumption of IDC. The DNP was found to be identical (m.p. and mixed m.p.) with the DNP of acetone. In similar experiments, with other alcohols, the yield of the DNP was in the range of 80-88% after recrystallization. The oxidation state of chromium in completely reduced reaction mixture, determined by iodometric titrations was 3.90 ± 0.10 .

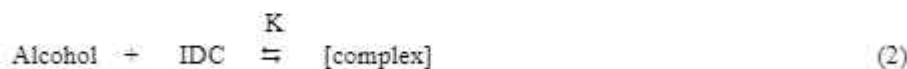
2.3 Kinetic Measurements: Reactions were carried out under pseudo-first-order conditions by keeping an excess ($\times 15$ or greater) of the alcohol over IDC. The solvent was DMSO, unless mentioned otherwise. All reactions were carried out in flasks blackened from the outside to prevent any photochemical reactions. The reactions were carried out at constant temperature (± 0.1 K) and were followed up to 80% of the extent of reaction, by monitoring the decrease in the concentration IDC spectrophotometrically at 361 nm. The pseudo-first-order rate constant, k_{obs} , was computed from the linear least-squares plot of $\log [\text{IDC}]$ versus time. Duplicate runs showed that the rate constants were reproducible to within $\pm 3\%$.

3. Results and Discussion: The rate and other experimental data were obtained for all the alcohols. Since the results are similar, only representative data are reproduced here.

3.1 Stoichiometry: The oxidation of alcohols by IDC leads to the formation of corresponding ketones. The overall reaction may be written as:



3.2 Rate-Laws: The reactions are of first order with respect to IDC. Further, the pseudo-first order rate constant, k_{obs} is independent of the initial concentration of IDC. The reaction rate increases with increase in the concentration of the alcohols but not linearly (Table 1). The figure 1 depicts a typical kinetic run. A plot of $1/k_{\text{obs}}$ against $1/[\text{Alcohol}]$ is linear ($r > 0.995$) with an intercept on the rate-ordinate. Thus, Michaelis-Menten type kinetics is observed with respect to the alcohols. This leads to the postulation of following overall mechanism (2) and (3) and rate law (4).



$$\text{Rate} = k_2 K [\text{Alcohol}] [\text{IDC}] / (1 + K [\text{Alcohol}]) \quad (4)$$

The dependence of reaction rate on the reductant concentration was studied at different temperatures and the values of K and k_2 were evaluated from the double reciprocal plots (Figure 2). The thermodynamic parameters of the complex formation and activation parameters of the decomposition of the complexes were calculated from the values of K and k_2 respectively at different temperatures (Tables 2 and 3).

3.3 Test for free radicals: The oxidation of 2-propanol by IDC₂ in an atmosphere of nitrogen, failed to induce the polymerization of acrylonitrile. Further, the addition of acrylonitrile had no effect on the rate (Table 1). Thus a one electron oxidation, giving rise to free radicals, is unlikely. To further confirm the absence of free radicals in the reaction pathway, the reaction was carried out in the presence of 0.05 mol dm⁻³ of 2,6-di-*t*-butyl-4-methylphenol (butylated hydroxytoluene or BHT). It was observed that BHT was recovered unchanged, almost quantitatively.

3.4 Kinetic isotope effect: To ascertain the importance of the cleavage of α-C-H bond in the rate-determining step, the oxidation of benzhydrol-α-d (PhCDOHPh) was studied. The oxidation of deuteriated benzhydrol exhibited a substantial primary kinetic isotope effect (Table 2).

3.5 Effect of hydrogen ions: The reaction is catalysed by hydrogen ions (Table 4). The hydrogen-ion dependence has the following form $k_{\text{obs}} = a + b [\text{H}^+]$. The values of *a* and *b*, for 2-propanol, are $4.56 \pm 0.15 \times 10^{-3} \text{ s}^{-1}$ and $7.90 \pm 0.25 \times 10^{-3} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ respectively ($r^2 = 0.9961$).

3.6 Effect of solvents: The oxidation of 2-propanol was studied in 19 different organic solvents. The choice of solvents was limited due to the solubility of IDC and its reaction with primary and secondary alcohols. There was no reaction with the solvents chosen. The kinetics was similar in all the solvents. The values k_2 at 308 K are recorded in Table 5.

The correlation between activation enthalpies and entropies of the oxidation of ten secondary alcohols is linear ($r = 0.9634$), indicating the operation of a compensation effect[13]. The value of the isokinetic temperature evaluated[14,15] from this plot is $522 \pm 51 \text{ K}$. However, according to Exner[16], an isokinetic relationship between the calculated values of activation enthalpies and entropies is often vitiated by random experimental errors. Exner suggested an alternative method for establishing the isokinetic relationship. Exner's plot between $\log k_2$ at 288 K and at 318 K was linear (slope = $0.8237 + 0.0183$; $r = 0.9980$; Figure 3). The value of isokinetic temperature evaluated from the Exner's plot is $631 \pm 78 \text{ K}$. The linear isokinetic correlation implies that all the alcohols are oxidized by the same mechanism and the changes in the rate are governed by changes in both the enthalpy and entropy of activation.

3.7 Solvent effect: The rate constants of the oxidation, k_2 , in eighteen solvents (CS₂ was not considered, as the complete range of solvent parameters was not available) were correlated in terms of the linear solvation energy relationship (LESR) of Kamlet and Taft[17] (5).

$$\log k_2 = A_0 + p\pi^* + b\beta + a\alpha \quad (5)$$

In this equation, π^* represents the solvent polarity, β the hydrogen bond acceptor basicities and α is the hydrogen bond donor acidity. A_0 is the intercept term. It may be mentioned here that out of the 18 solvents, 12 have a value of zero for α . The results of correlation analyses in terms of equation (3), a biparametric equation involving π^* and β , and separately with π^* and β are given below as equations (6) - (9).

$$\log k_2 = -3.72 + 1.61 (\pm 0.21) \pi^* + 0.17 (\pm 0.17) \beta + 0.04 (\pm 0.16) \alpha \quad (6)$$

$$R^2 = 0.8427; \quad \text{sd} = 0.19; \quad n = 18; \quad \psi = 0.43$$

$$\log k_2 = -3.73 + 1.62 (\pm 0.19) \pi^* + 0.15 (\pm 0.16) \beta \quad (7)$$

$$R^2 = 0.8420; \quad \text{sd} = 0.18; \quad n = 18; \quad \psi = 0.42$$

$$\log k_2 = -3.70 + 1.66 (\pm 0.19) \pi^* \quad (8)$$

$$r^2 = 0.8320; \text{ sd} = 0.18; \text{ n} = 18; \psi = 0.42$$

$$\log k_2 = -2.79 + 0.44 (\pm 0.36) \beta \quad (9)$$

$$r^2 = 0.0881; \text{ sd} = 0.43; \text{ n} = 18; \psi = 0.98$$

Here n is the number of data points and ψ is the Exner's statistical parameter[18].

Kamlet's¹⁷ triparametric equation explains *ca.* 84% of the effect of solvent on the oxidation. However, by Exner's criterion[18] the correlation is not even satisfactory (cf. equation 6). The major contribution is of solvent polarity. It alone accounted for *ca.* 83% of the data. Both β and α play relatively minor roles.

The data on the solvent effect were analysed in terms of Swain's[19] equation (10) of cation- and anion-solvating concept of the solvents also.

$$\log k_2 = aA + bB + C \quad (10)$$

Here A represents the anion-solvating power of the solvent and B the cation-solvating power. C is the intercept term. $(A + B)$ is postulated to represent the solvent polarity. The rates in different solvents were analysed in terms of equation (8), separately with A and B and with $(A + B)$.

$$\log k_2 = 0.75(\pm 0.05) A + 1.69 (\pm 0.03) B - 3.95 \quad (11)$$

$$R^2 = 0.9936; \text{ sd} = 0.04; \text{ n} = 19; \psi = 0.08$$

$$\log k_2 = 0.51 (\pm 0.56) A - 2.79 \quad (12)$$

$$r^2 = 0.0467; \text{ sd} = 0.45; \text{ n} = 19; \psi = 1.00$$

$$\log k_2 = 1.64 (\pm 0.14) B - 3.71 \quad (13)$$

$$r^2 = 0.8936; \text{ sd} = 0.15; \text{ n} = 19; \psi = 0.33$$

$$\log k_2 = 1.38 \pm 0.12 (A + B) - 3.92 \quad (14)$$

$$r^2 = 0.8936; \text{ sd} = 0.15; \text{ n} = 19; \psi = 0.33$$

The rates of oxidation of 2-propanol in different solvents showed an excellent correlation in Swain's equation (cf. equation 11) with the cation-solvating power playing the major role. In fact, the cation-solvation alone account for *ca.* 99% of the data. The correlation with the anion-solvating power was very poor. The solvent polarity, represented by $(A + B)$, also accounted for *ca.* 88% of the data. In view of the fact that solvent polarity is able to account for *ca.* 88% of the data, an attempt was made to correlate the rate with the relative permittivity of the solvent. However, a plot of $\log k_2$ against the inverse of the relative permittivity is not linear ($r^2 = 0.5098$; $\text{sd} = 0.32$; $\psi = 0.72$).

3.8 Correlation analysis of reactivity: The rates of oxidation of alcohols failed to yield any significant correlation separately with Taft's[20] σ^* and E_s values as equations (15) and (16).

$$\log k_2 = -1.72 (\pm 0.14) \Sigma \sigma^* - 2.19 \quad (15)$$

$$r^2 = 0.9641; \text{ sd} = 0.16; \text{ n} = 8; \psi = 0.20; \text{ T} = 298 \text{ K}$$

$$\log k_2 = -1.23 (\pm 0.96) \Sigma E_s - 2.79 \quad (16)$$

$$r^2 = 0.2131; \text{ sd} = 0.74; \text{ n} = 8; \psi = 0.93; \text{ T} = 298 \text{ K}$$

The rates were, therefore, correlated in terms of Pavelich-Taft's[21] dual substituent -parameter equation (17).

$$\log k = \rho^* \sum \sigma^* + \delta \sum E_s + \log k_0 \quad (17)$$

The values of the substituent constants were obtained from the literature[20]. The correlations are excellent, reaction constants being negative (Table 6). There is no significant collinearity ($r^2 = 0.2322$) between σ^* and E_s of the eight substituents.

The negative polar reaction constant indicates an electron-deficient carbon centre in the transition state of the rate-determining step. The negative steric reaction constant points to a steric acceleration of the reaction. This may be explained on the basis of the high ground state energy of the sterically crowded alcohols. Since the crowding is relieved in the product, ketone, as well as in the transition state leading to it, the transition state energies of the crowded and un-crowded alcohols do not differ much and steric acceleration, therefore, results. The faster oxidation of 1-phenylethanol and benzhydrol may well be due to the stabilization of the electron-deficient carbon centre in the transition state by phenyl group by resonance.

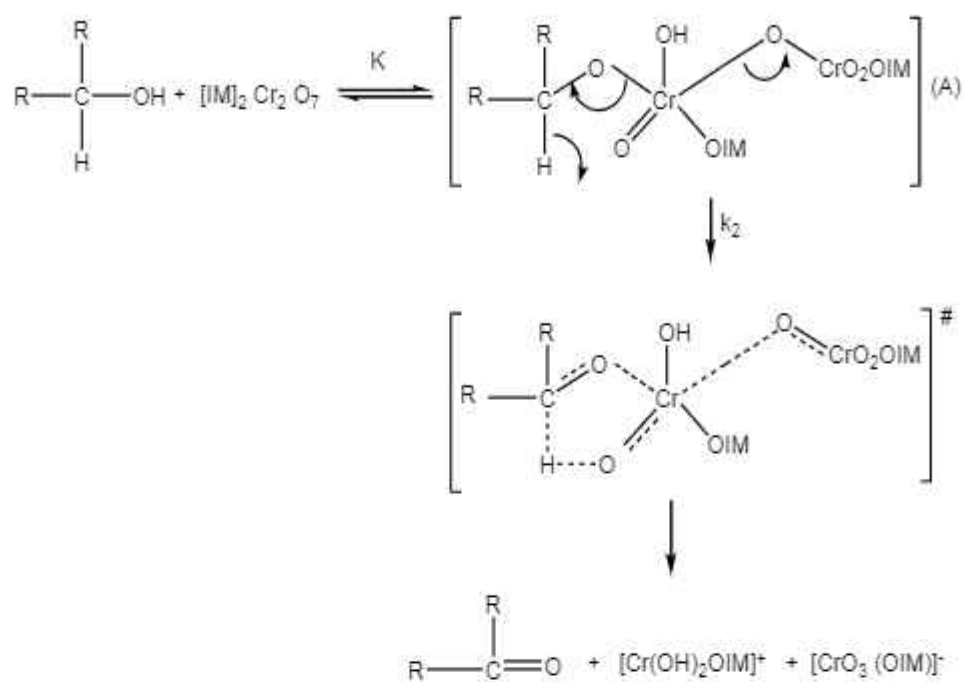
4. Mechanism

The presence of a substantial primary kinetic isotope effect ($k_H/k_D = 5.79$ at 298 K), confirms that the α -C-H bond is cleaved in the rate-determining step. The large negative value of the polar reaction constant together with the substantial deuterium isotope effect indicates that the transition state approaches a carbocation in character. Hence, transfer of a hydride ion from the alcohol to the oxidant is suggested. The hydride ion transfer mechanism is also supported by major role of cation-solvating power of the solvents. The hydride-ion transfer may proceed by either an acyclic bimolecular reaction or may involve a cyclic symmetrical transition state *via* a chromate ester. Kwart and Nickle[22] have shown that a study of the dependence of the kinetic isotope effect on temperature can be gainfully employed to resolve this problem. The data for protio- and deuterio-benzhydrols, fitted to the familiar expression $k_H/k_D = A_H/A_D \exp(E_s/RT)$ show a direct correspondence with the properties of a symmetrical transition state[23,24] in which the activation energy difference (E_s) for k_H/k_D is equal to the zero-point energy difference for the respective C-H and C-D bonds (≈ 4.5 kJ/mol) and the frequency factors and the entropies of activation of the respective reactions are nearly equal. Bordwell[25] has documented a very cogent evidence against the occurrence of concerted one-step biomolecular processes by hydrogen transfer and it is evident that in the present studies also the hydrogen transfer does not occur by an acyclic biomolecular process. It is well-established that intrinsically concerted sigmatropic reactions, characterised by transfer of hydrogen in a cyclic transition state, are the only truly symmetrical processes involving a linear hydrogen transfer[26]. Littler[27] has also shown that a cyclic hydride transfer, in the oxidation of alcohols by Cr(VI), involves six electrons and, being a Huckel-type system, is an allowed process. Thus, a transition state having a planar, cyclic and symmetrical structure can be envisaged for the decomposition of the ester intermediate. Hence, the overall mechanism is proposed to involve the formation of a chromate ester in a fast pre-equilibrium step and then a decomposition of the ester in a subsequent slow step *via* a cyclic concerted symmetrical transition state leading to the product (Schemes 1 and 2). The observed negative value of entropy of activation also supports a polar transition state.

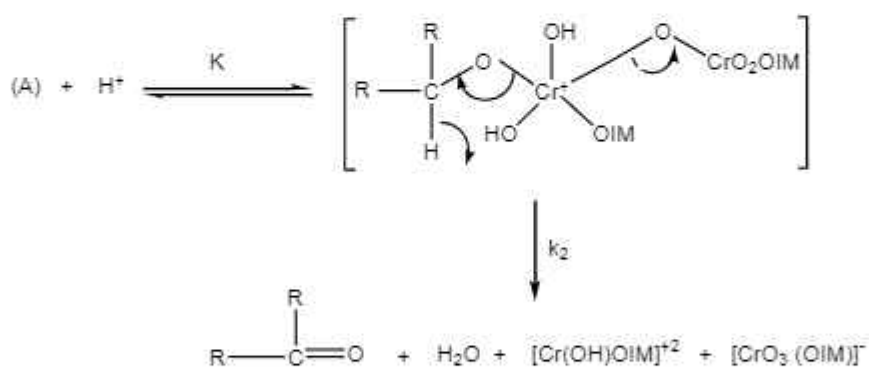
The observed negative value of entropy of activation also supports the proposed mechanism. As the charge separation takes place in the transition state, the charged ends become highly solvated. This results in an immobilization of a large number of solvent molecules, reflected in the loss of entropy[28].

5. Acknowledgements

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Acid-independent Path - Scheme - 1



Acid-dependent Path - Scheme - 2

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TABLE 1. RATE CONSTANTS FOR THE OXIDATION OF 2-PROPANOL BY IDC AT 318 K

10^3 [IDC] mol dm ⁻³	[2-Propanol] mol dm ⁻³	$10^4 k_{obs}$ s ⁻¹
1.00	0.10	4.48
1.00	0.20	7.20
1.00	0.40	10.3
1.00	0.60	12.1
1.00	0.80	13.2
1.00	1.00	14.0
1.00	1.50	15.2
1.00	3.00	16.6
2.00	0.20	7.65
4.00	0.20	7.29
6.00	0.20	7.47
8.00	0.20	7.02
1.00	0.40	10.8*

* contained 0.001 M acrylonitrile

TABLE 2. FORMATION CONSTANTS AND THERMODYNAMIC PARAMETERS FOR THE ALCOHOLS – IDC COMPLEXES

Alcohol	K (dm ³ mol ⁻¹)				$-\Delta H^\ddagger$ (kJ mol ⁻¹)	$-\Delta S^\ddagger$ (J mol ⁻¹ K ⁻¹)	$-\Delta G^\ddagger$ (kJ mol ⁻¹)
	288 K	298 K	308 K	318 K			
Me	5.63	4.80	4.05	3.24	16.4±0.7	34±2	6.34±0.6
Et	6.03	5.25	4.40	3.63	15.4±0.6	30±2	6.55±0.5
n-Pr	5.94	5.15	4.30	3.51	15.8±0.7	32±2	6.50±0.5
i-Pr	5.76	4.92	4.10	3.33	16.4±0.6	34±3	6.40±0.5
i-Bu	5.85	5.06	4.20	3.42	16.1±0.7	33±2	6.45±0.5
Bu	6.12	5.33	4.52	3.69	15.3±0.7	30±2	6.59±0.6
ClCH ₂	5.58	4.75	3.92	3.15	17.0±0.7	36±2	6.30±0.5
MeOCH ₂	5.67	4.88	4.05	3.26	16.5±0.7	35±2	6.37±0.6
Ph	5.90	5.10	4.32	3.47	15.8±0.8	32±3	6.49±0.6
Benzhydrol	5.70	4.86	4.08	3.24	16.7±0.8	35±3	6.37±0.6
Benzhydrol- α -D	5.92	5.13	4.32	3.49	15.8±0.8	32±2	6.50±0.6

TABLE 3. RATE CONSTANTS AND ACTIVATION PARAMETERS FOR THE ALCOHOLS – IDC COMPLEXES

Alcohol	$10^4 k_2 / (\text{dm}^3 \text{mol}^{-1} \text{s}^{-1})$				ΔH^\ddagger	$-\Delta S^\ddagger$	ΔG^\ddagger
	288 K	298 K	308 K	318 K	(kJ mol ⁻¹)	(J mol ⁻¹ K ⁻¹)	(kJ mol ⁻¹)
Me	18.9	42.3	88.2	180	54.6±0.1	108±1	86.6±0.1
Et	30.6	66.6	135	261	51.8±0.2	113±1	85.5±0.2
n-Pr	49.5	99.9	189	351	47.1±0.2	126±1	84.4±0.1
i-Pr	78.3	153	279	495	44.2±0.2	132±1	83.4±0.2
i-Bu	117	207	351	585	38.3±0.1	149±1	82.6±0.1
Bu	54.9	108	207	378	46.5±0.2	127±1	84.2±0.1
ClCH ₂	0.43	1.13	2.79	6.75	67.2±0.3	95±1	95.5±0.3
MeOCH ₂	2.97	7.65	17.1	36.0	61.2±0.6	100±2	90.9±0.5
Ph	126	234	441	819	45.0±0.7	125±2	82.3±0.5
Benzhydrol	135	261	477	891	45.2±0.5	124±1	82.1±0.4
Benzhydrol- <i>o</i> -D	22.3	45.1	86.1	171	48.9±0.6	126±2	86.4±0.5
k_H/k_D	6.05	5.79	5.54	5.21			

TABLE 4. DEPENDENCE OF THE REACTION RATE ON HYDROGEN-ION CONCENTRATION

[IDC] = 0.001 mol dm ⁻³ ; [2-propanol] = 0.10 mol dm ⁻³ ; Temp. = 318 K						
[H ⁺]/mol dm ⁻³	0.10	0.20	0.40	0.60	0.80	1.00
$10^4 k_{\text{obs}}/\text{s}^{-1}$	5.22	6.39	7.74	9.09	10.8	12.6

TABLE 5. EFFECT OF SOLVENTS ON THE OXIDATION OF 2-BUTANOL BY IDC AT 308 K

Solvents	K (dm ⁻³ mol ⁻¹)	$10^4 k_{\text{obs}}$ (s ⁻¹)	Solvents	K (dm ⁻³ mol ⁻¹)	$10^4 k_{\text{obs}}$ (s ⁻¹)
Chloroform	5.55	41.7	Toluene	4.89	12.0
1,2-Dichloroethane	5.68	51.3	Acetophenone	5.65	54.9
Dichloromethane	6.12	39.8	THF	5.99	22.4
DMSO	4.40	135	t-Butylalcohol	5.77	18.2
Acetone	5.89	44.7	1,4-Dioxane	5.55	19.5
DMF	5.85	63.1	1,2-Dimethoxyethane	5.80	10.2
Butanone	6.03	32.4	CS ₂	5.60	5.25
Nitrobenzene	6.21	49.0	Acetic Acid	5.75	9.12
Benzene	5.89	15.5	Ethyl Acetate	5.96	14.4
Cyclohexane	5.88	1.51			

TABLE 6. TEMPERATURE DEPENDENCE OF REACTION CONSTANTS

Temp/ K	$-\rho^*$	$-\delta$	r^2	Sd	ψ
288	1.71 ± 0.01	0.63 ± 0.01	0.9998	0.013	0.02
298	1.62 ± 0.01	0.53 ± 0.02	0.9999	0.004	0.01
308	1.53 ± 0.01	0.44 ± 0.01	0.9989	0.002	0.04
318	1.44 ± 0.01	0.36 ± 0.01	0.9999	0.007	0.01

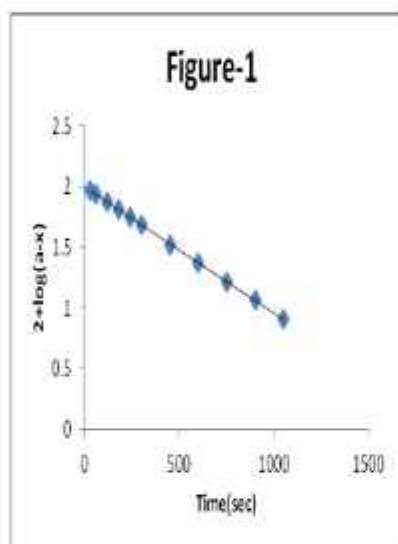


Figure 1
Oxidation of 2-propanol by IDC: A typical kinetic run

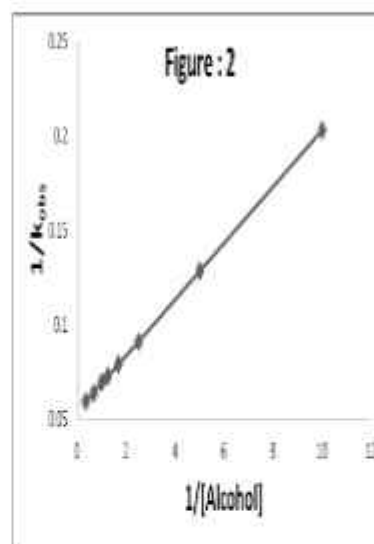


Figure 2
Oxidation of 2-propanol by IDC: A double reciprocal plot

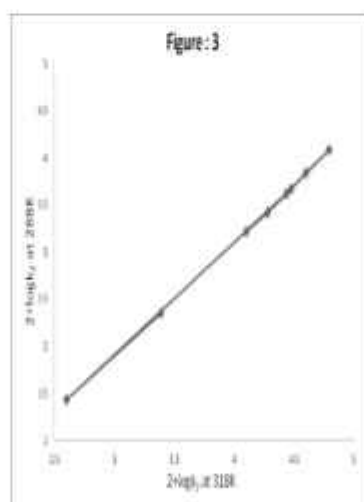


Figure 3
Exner's Isokinetic Relationship in the oxidation of Alcohols by IDC



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Implementation of Genetic Engineering and Novel Omics Approaches to Enhance Bioremediation: A Focused Review

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Abstract

Bioremediation itself is considered to be a cost effective soil clean-up technique and preferred over invasive physical and chemical treatments. Besides increasing efficiency, application of genetic engineering has led to reduction in the time duration required to achieve remediation, overcoming the so called 'Achilles heel' of Bioremediation. Omics technologies, namely genomics, transcriptomics, proteomics, and metabolomics, are being employed extensively to gain insights at genetic level. A wise synchronised application of these approaches can help scrutinize complex metabolic pathways, and molecular changes in response to heavy metal stress, and also its fate i.e., uptake, transport, sequestration and detoxification. In the present review, an account of some latest achievements made in the field is presented.

Keywords Genetic engineering · Multi-omics · Bioremediation

Introduction

Exclusion or alleviation of the lethal effects of complex chemical compounds added to the surrounding environment by anthropogenic activities depends mainly on the biological systems. Bioremediation involves multidisciplinary approach including biosorption (metal sorption to cell surface by physicochemical systems), bioaugmentation (addition of microbes that have catabolic genes), biostimulation (organisms with elevated degradation capabilities are utilized to inoculate the polluted site), mycoremediation (use of fungi to solubilize, transform or uptake metal contaminants) and phytoremediation (plants are used to accumulate and metabolize toxic compounds in polluted areas). In current scenario, bioremediation via microbiological processes have the upper hand in degrading huge variety of pollutants such as heavy metal, petroleum, persistent organic pollutants, xenobiotics, and radionuclides, in terms of sustainability

and unproblematic in-situ applicability. Recent advances in systemic biology and gene editing techniques for e.g., TALEN, CRISPR/Cas and ZFN have created the possibility to modify microbes to catabolize specific toxic compounds and eventually be applied in bioremediation process to tackle environment pollution challenge on a global scale. Omics studies (genomics, transcriptomics, proteomics, and metabolomics) assist the microbial systems biology research for exploring regulations at the molecular level for bioremediation and have introduced a wide perceptive of specific genes and proteins of microorganism, involved in degradation of pollutants in contaminated sites (Jaiswal et al. 2019; Pant et al. 2021).

Bioremediation Using Genetically Engineered Bacteria

Bacteria play vital role in treating the soil contaminated with toxic chemical pollutants via three main processes; mobilization, transformation and detoxification. The study of these mechanisms may unravel genetic factors controlling them and thereby could also be manipulated to be an asset in bioremediation processes overtime. A genetically engineered *Rhodospseudomonas palustris* strain was constructed to express mercury transport system and metallothionein concurrently for exclusion of mercury from heavy metal

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wastewater. The recombinant bacterium showed stronger resistance to poisonous Hg^{2+} and three times elevated Hg^{2+} binding capacity as compared to wild type *R. palustris* (Deng and Jia 2011). Similarly, *Escherichia coli* recombinant strain derived by inserting metallothionein (MT) and two nickel-affinity transmembrane proteins NixA and NisA from *Helicobacter pylori* and *Staphylococcus aureus* respectively, showed enhanced tolerance and bioaccumulation of Ni^{2+} (Deng et al. 2013). A wild type *Bacillus subtilis* 168 does not possess methylation and volatilization properties, however, when arsenite S-adenosylmethionine methyltransferase gene of thermophilic algae (*Cyanidioschyzon merolae*) was inserted into it, the genetically engineered *B. subtilis* 168 bacterium successfully convert the inorganic arsenic via methylation into dimethylarsenate and trimethylarsine oxide, and volatilize large quantities of dimethylarsine and trimethylarsine (Huang et al. 2015).

Other than these, the use of biosurfactant (microbial biopolymer) in eliminating heavy metals from polluted sites is emerging as a promising technique. Biosurfactants of microbial origin are metal-complexing agents that form stable complexes with metal ions. A biosurfactant-producing *Bacillus cereus* NWUAB01 strain with metal removal efficiency of 69%, 54% and 43% for Pb, Cd and Cr respectively, was isolated from a mining soil that can be utilized for biotechnological applications. Genomic study revealed presence of genes accountable for metal transport or resistance and clusters of biosynthetic genes implicated in the production of a variety of secondary metabolites (Ayangbenro and Babalola 2020). Many strains of *Pseudomonads* are known to degrade both aliphatic and aromatic hydrocarbons as the plasmids responsible for hydrocarbon degradation contain genes to carry out the degradation. *Pseudomonas putida* strain BS3701 isolated from coke by-product waste contaminated soil was found capable of degrading crude oil and polycyclic aromatic hydrocarbon. Its genome sequencing revealed the presence of plasmids pBS1141, harbouring genes for naphthalene degradation and helper plasmid pBS1142. The genome sequencing data of this strain will give insights of the metabolic capabilities of *Pseudomonas* strains that can be utilized for bioremediation purpose (Filonov et al. 2020). *Pseudomonas putida* strain KT2440 was genetically engineered using genome-editing method with four pesticide-degrading genes (for simultaneous degradation of organophosphates, pyrethroids, and carbamates) and *vgb* (for superior oxygen-sequestering ability). The resulting recombinant strain, *P. putida* KTUe, showed promising results and may serve as a potential candidate for remediation of multiple pesticides contaminated soil *in situ*. This research highlights the influence of synthetic biology in enhancing the degradation ability of natural degraders (Gong et al. 2018). Similarly, the genetically

engineered bacterium, *Pseudomonas putida* MC4-5222, obtained by introducing haloalkane dehalogenase gene (*dhaA31*) with improved 1,2,3-Trichloropropane (TCP) degradation activity into DCP (2,3-dichloro-1-propanol)-degrading bacterium *P. putida* MC4 showed successful conversion of all organic chlorine into chloride (Samir et al. 2014). A new metabolically multitalented, biosurfactant-producing PGPR, *Pseudomonas rhizophila* S211, showing bioremediation potentialities was isolated from artichoke field contaminated with pesticide. *P. rhizophila* S211 could be used as a promising tool for bioremediation of pesticide-polluted soils due to its emulsification actions and elevated capacity in solubilization augmentation of pesticides (Hassen et al. 2018). Similarly, a hydrocarbon-degrading and biosurfactant producing endophytic bacterium, *Pseudomonas aeruginosa* L10, isolated from the roots of *Phragmites australis*, has shown potential in degradation of C10–C26 n-alkanes and polycyclic aromatic hydrocarbons (PAHs) like pyrene, phenanthrene, and naphthalene. Genome analysis revealed presence of genes (monooxygenase, dioxygenase, alcohol dehydrogenase, and aldehyde dehydrogenase) related to petroleum hydrocarbon degradation, and a gene cluster implicated in rhamnolipids (*rhlABRI*) biosynthesis that is accountable for the biosurfactant activity (Wu et al. 2018).

The capability to degrade a wide variety of hydrocarbons has been shown by different species of the *Acinetobacter* genus. Recently, *Acinetobacter calcoaceticus* strain CA16 has shown prospects for bioremediation of aliphatic alkane hydrocarbons in diesel oil polluted sites. Various diesel-degrading genes like *alkM* and *xcpR* were revealed to be activated in CA16 (Ho et al. 2020). *Burkholderia vietnamiensis* G4 has biodegradation potential and is used as a bioremediation model due to its ability to degrade highly harmful pollutants such as naphthalene, benzene, o-cresol, p-cresol, phenol, chloroform, toluene, and benzo(a)pyrene. The expression of *B. vietnamiensis* G4 bacterium genes was evaluated under PAH benzo(a)pyrene exposure, and a total of 156 differentially expressed genes were observed, out of which 88 genes showed higher expression signifying the prospect of several metabolic pathways of degradation of PAH benzo(a)pyrene in this bacteria (Cauduro et al. 2020). The genetically engineered *Cupriavidus necator* strain JMP134-ONP obtained after inserting an *onpABC* gene cluster (ortho-nitrophenol degradation operon) into *Cupriavidus necator* JMP134 has shown the capability to degrade two isomers-ortho-nitrophenol and meta-nitrophenol, concurrently (Hu et al. 2014). Some other bacterial strains i.e., *Enterobacter cloacae* HS32, *Brevibacillus reuszeri* HS37, *Stenotrophomonas* sp. HS16, *Acinetobacter junii* HS29, *Enterobacter aerogenes* HS39 and *Enterobacter asburiae* HS22 have been also used to formulate consortia for PAH degradation and metal removal (Sarma et al. 2019).

Mycoremediation Using Genetically Engineered Fungi

Fungi also inhabit the rhizosphere and a lot of different fungal species live both endophytically and symbiotically playing a vital role in bioremediation. These organisms adapt to live in a toxic environment via genetic and morphological modifications. The growth of fungal hyphae also helps in the transport of bacteria to the toxic site at a distance enhancing the rate of bioremediation. The fungi *Pythium ultimum* transported the *Pseudomonas putida* PpG7 (NAH7) strain to degrade phenanthrene present in the soil (Wick et al. 2007). Fungi as a natural source organism for bioremediation have also been reported in various studies. The filamentous fungal species of *Aspergillus* has been reported to be able to degrade PAHs, chlorophenols and aliphatic hydrocarbons. A similar role has also been reported for *Penicillium* species (Li et al. 2020). Other examples of pollutants include Anthracene, removed by *Irpexlacteus* and *Pleurotus ostreatus* fungus (Drevinskas et al. 2016); Pyrene by *Ganoderma lucidum* (Agrawal et al. 2018) and Chrysene by *Polyporus* spp. (Hadibarata et al. 2009). These fungal groups have also been able to degrade toxic metals with high bioremediation efficiency, for example, *Pleurotus ostreatus* (white rot fungus) is known to aid in bioremediation by degradation of crude oil and toxic metals such as: Cd, Cu, Mn, Ni and Pb with bioremediation efficiency within the range of 28.2%–75.9% (Anacleto et al. 2017). External factors that affect the bioremediation process include change in the temperature and pH of the soil whereas internal factors affecting bioremediation are the modifications in the cellular environment, such as changes in the expression pattern of enzymes such as laccases, lignin peroxidases and peroxidases-manganese, and the synthesis of metabolites (Deshmukh et al. 2016a; Li et al. 2020).

Genome analysis of fungal strains has provided new perspective to engineer and use them as biotechnological tools. Metagenomic analysis has helped to identify different taxa of fungi in diverse ecosystems which are being researched for their detoxification potential. A few examples include the genetically engineered *Pichia pastoris* fungus containing the yeast laccase gene (*YILac*) from the fungus *Yarrowia lipolytica* which degrades phenolic compounds (Kalyani et al. 2015). Similarly, the laccase gene from *Phanerochaete flavido-alba* was expressed in *Aspergillus niger* which was reported to degrade synthetic textile dyes such as acid red and Brilliant blue R (Benghazi et al. 2014). A recombinant strain of yeast *S. cerevisiae* was reported that was able to degrade dioxins by expressing a mammalian cytochrome *CYP1A* gene. The *CYP1A* construct had the genes *CYP1A1* and *CYP1A2*

from the rat which were fused with the NADPH-P450 reductase gene from the yeast. This recombinant strain was able to degrade dibenzo-p-dioxin, 1-monochlorodibenzo-p-dioxin and other dioxins except 2,3,7,8-tetra-CDD (Sakaki et al. 2002). Later this was solved by using site directed mutagenesis and modified the binding pocket of the *CYP1A1* enzyme and suggested that this modification increased the rate of 2,3,7,8-tetra-CDD biodegradation (Shinkyō et al. 2003). A recombinant strain of *Fusarium* generated by parasexual hybridisation has also been reported to be effective against DDT degradation (Mitra et al. 2001). Thus, fungi have been used as a bioremediation tool from a long time, both in its naturally occurring form and as a genetically engineered organism.

Table 1 outlines an exhaustive list of microorganisms that may contribute to the future design of rational strategies for bioremediation of contaminated environment.

The Omics Insight of Bioremediation

With advancement in Next-generation sequencing (NGS), new avenues have been discovered in bioremediation. It is now possible to get an insight into key biodegradative pathways of environmentally important microbes. Techniques like metagenomics, meta-transcriptomics, meta-proteomics, and other omic methods (Fig. 1) have increased our understanding of how microbial communities are structured, how do they interact with each other and their counter organisms and how do they participate in a complex interaction network between the plants, microbes and the environment.

The microbial communities evolve and change their structure at a high rate. Environmental factors are the main drivers behind this dynamic behaviour. Another factor is the change in the microenvironmental conditions, which change due to the process of biodegradation. As the pollutants are decomposed, they are converted into less complex molecules with variable chemical properties thus, changing the pH of the soil. This minute change prohibits the growth of many microbes but simultaneously promotes the growth of some other microbial species ("Microb. Biodegrad. From Omi. to Funct. Appl.," 2016).

Metagenomics has been employed for better understanding of the genome organisation of the microbial communities inhabiting the rhizosphere and to identify the genes in different microbial species that participate in bioremediation. Since microbes reside in communities, metagenomics find its application as we can't separate one microbe from another. Bioremediation as a process is wholly affected by communities residing in the contaminated matrix and the potential of certain microbial species may be synergistically or antagonistically related with the other. Once a metagenomic library is sequenced, the reads are aligned

Table 1 List of some microorganisms recently investigated for their potential use in remediation

Microorganisms	Compound/heavy metal	Properties/application	References
<i>Rhodococcus</i> sp. IN306	Polychlorinated biphenyls (PCBs)	Contains genes encoding for the enzyme degrading hydrocarbon	Steliga et al. (2020)
<i>Mycobacterium frederiksbergense</i> IN53	Total petroleum hydrocarbons (TPH)	Contains genes encoding for the enzyme degrading hydrocarbon	Steliga et al. (2020)
<i>Pseudomonas</i> sp. (strain CB-3) and <i>Comamonas</i> sp. (strain CD-2)	PCBs	CB-3 genome contain gene cluster of biphenyl metabolism. CD-2 possess a dechlorination and protocatechuate (PCA) metabolic gene cluster	Xing et al. (2020)
<i>Rhodococcus erythropolis</i> strain CD 167	Total petroleum hydrocarbon	Elevated expression of genes involved in TPH degradation	Pacwa-Pfociniczak et al. (2019)
Marine bacterium <i>Achromobacter</i> sp. HZ01	Petroleum hydrocarbon	Degrades <i>n</i> -alkanes and aromatic compounds via the terminal oxidation pathway and catechol pathway respectively; possesses genes involved in biosurfactant synthesis.	Hong et al. (2017)
<i>Alphaproteobacterium Rhizobium</i> sp. NT-26	Arsenic	Contain various genes that enable it to metabolize arsenite	Andres et al. (2013)
<i>Cupriavidus necator</i> strain JMS34	PCBs	Genetically modified strain with insertion of <i>bph</i> locus of <i>Burkholderia xenovorans</i> LB400 (encodes PCB degradation pathway)	Saavedra et al. (2010)
<i>Deinococcus radiodurans</i>	Uranium	The gene <i>phoN</i> upon overexpression of periplasmic nonspecific acid phosphatase degrade Uranium at polluted sites.	Appukuttan et al. (2011)
<i>Trichoderma atroviride</i>	Dichlorvos pesticide	Hygromycin B phosphotransferase (<i>hph</i>) gene from the plasmid pV2 was used to transform the T23 strain improving degradation capability.	Tang et al. (2009)
<i>Paecilomyces lilacinus</i> NH1	Cadmium	Improved Cd phytoextraction and antioxidative defense in <i>Solanum nigrum</i> L.	Gao et al. (2010)

to identify the genes and the microbial species present in the sample. Unfortunately, the limitation of metagenomics is that it cannot recognise whether the identified genes are expressed or not. That is why, metagenomic analysis is often coupled with meta-transcriptomics, i.e., the sequencing of the mRNA of the microbial community to understand the expression pattern of the genes that are up or downregulated upon changes in the microenvironment (Silva et al. 2012; Suenaga et al. 2007). Although, meta-transcriptomic is not widely employed due to mRNAs being highly unstable, but meta-proteomics is another technique that is used. Meta-proteomics help to identify all the expressed proteins in a sample at a given time under specific conditions (Szewczyk and Kowalski 2016a). Again, meta-proteomics alone cannot help to understand the complexity of microbial bioremediation and thus, interactomics and metabolomics are also integrated to understand the processes and pathways. Therefore, the

functional characterisation of the genes using an integrated approach of multi-meta-omics and Gene Ontology (GO) tools is the preferred approach to develop a better understanding of the molecular mechanisms involved in multipartite interactions shaping rhizosphere bioremediation.

Since meta-transcriptomics cannot always be employed as a choice of method therefore another approach of plasmid transformation is also used. In this process, the plasmid of the bacterial cells is modified to contain the metagenomic library and upon cell growth, they are tested for pollutant degradation using a chemical reaction. A study conducted by Silva et al. (2012) on petroleum refinery wastewater sample identified the group of bacteria belonging to the genera: *Comamonas*, *Diaphorobacter*, *Pseudomonas*, and *Thauera* containing genes that degrade benzoate, biphenyl compounds, naphthalene, phenol, and toluene. This suggests that a diverse variety of novel genes could possibly be

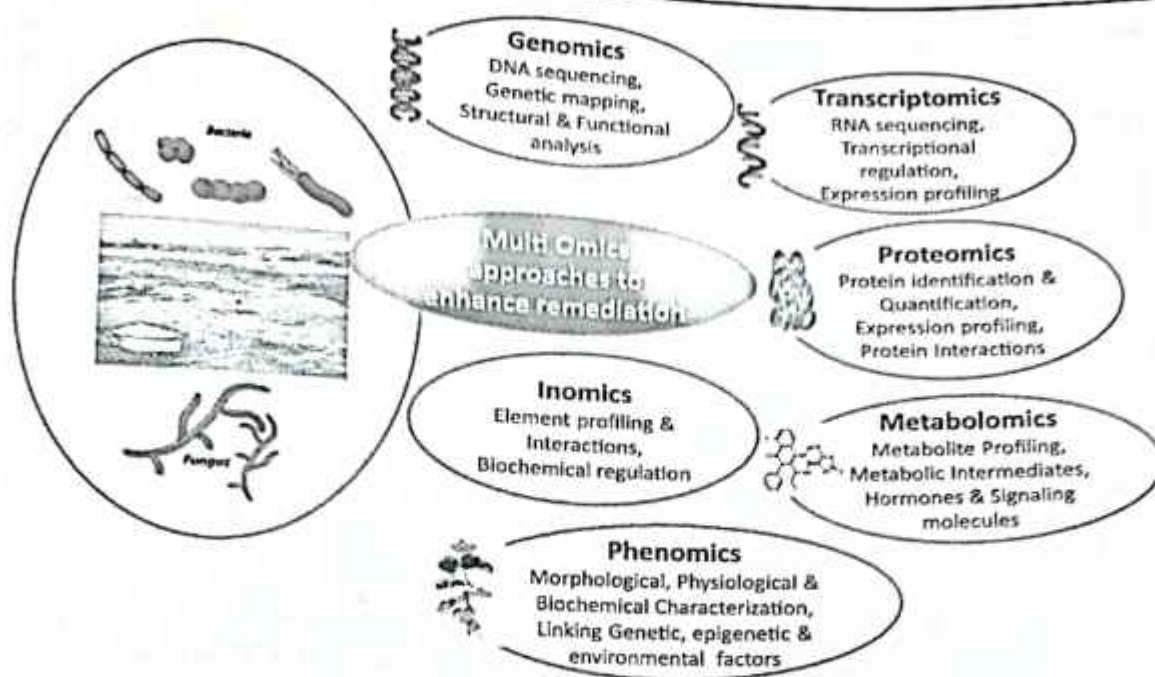


Fig. 1 Omics techniques and their components

present that might be able to degrade other organic compounds and help in bioremediation (Silva et al. 2012). In order to identify novel genes, methods such as BLAST (Basic Local Alignment Search Tool) could be used against databases of known reference genome assembly and pathway information's, such as KEGG (Kyoto Encyclopedia of Genes and Genomes) or other databases like MG-RAST (metagenomics Rapid Annotation using Subsystem Technology) (Meyer et al. 2008). Another challenge that is often faced in metagenome analysis is the identification of microbial species. In the same study, it was also reported that the gene *xylE* coding for catechol 2,3-dioxygenase was identified which shared strong homology with two bacterial groups, namely, *Pseudomonas* and *Thermomicrobium* but couldn't be classified as belonging to either (Silva et al. 2012). A study was conducted to understand the expression of four genes participating in biodegradation of aromatic compounds. Using real-time qPCR methods and comparative metagenomics, the genes *dmpB*, *tcbC*, *xylE*, and the genes coding for dioxygenases were identified (Yadav et al. 2015). A similar methodology can also be employed to identify new genes involved in heavy metal biodegradation and in other microbes such as fungi. A study employing meta-transcriptomic approach to understand the soil microbial communities and their role in biodegradation of phenanthrene, suggested that the genes associated with metabolism of aromatic compounds in both, bacteria and fungi are upregulated (de Menezes et al. 2012). Similarly, a study identified that the genes *bamC*, *bamD*,

bzlA and *ubiD*-like genes are expressed in *Clostridiales* bacterial species if they encounter benzene in their environment (Luo et al. 2014). Though, each of the omics approach has its own limitations, therefore more advancement in this direction is providing new answers to the questions of the microbial world. One such advancement is the single cell meta-transcriptomics. A study used single cell meta-genomic and meta-transcriptomic approach to understand the interactions among the microbial community to better understand the alkane degradation (Embree et al. 2014).

Despite their limitations, meta-genomics is a very powerful technique to identify the microbes in a complex environment of multiple interactions and to understand their phylogeny. Since genes such as 16 S rRNA and the recombination genes such as *recA* are highly conserved across various microbes, they seem to be the choice of phylogenetic biomarkers for microbe identification (Ziembinska-Buczynska 2016). It has been highlighted in various studies requiring de novo genome assembly that 16 S rRNA sequencing using high throughput shotgun sequencing has aided in the identification of various microbes available in the polluted environment. Other phylogenetic markers used for bacterial and fungal recognition are 23 S rRNA and 18 S rRNA respectively (Ziembinska-Buczynska 2016). Apart from coding, sometimes non-coding genes are also used as biomarkers to identify the microbial species. An example of fungal non-coding biomarker genes is *ITS1* and *ITS2* (internal transcribed spacer) (Embong et al. 2008). These

genes are located between the 18 and 5.8 S, and between 5.8 and 28 S rRNA genes. Similarly, for bacterial identification at community and species level, the *16-23 S rDNA ISR* (induced systemic resistance) gene is used as a phylogenetic biomarker (Liu et al. 2012; Ruiz et al. 2000).

Meta-genomics and transcriptomics have their own relevance, but the actual picture of the metabolic pathways is painted by the proteins and thus, meta-proteomics provide more information than meta-genomic and meta-transcriptomics alone. This is because the presence of specific proteins, their interactions and involvement in various pathways suggests a better understanding of their function in biodegradation. A study identified more than 500 proteins in the soil samples, suggesting a direct method of protein identification in a metaproteome (Chourey et al. 2010). Another study identified 1116 proteins which were further classified into various groups specific to the sample type (Guazzaroni et al. 2013). Meta-proteomics approach provides a deeper insight to the biodegradation process, but it suffers the challenge of data analysis. Integration of protein-protein, protein-metabolite, gene interaction networks could provide more details into pathway mapping for biodegradation and could shed light onto new aspects of microbial bioremediation.

Other fields such as metabolomics deal with the study of the metabolic flux in a sample. The meta-metabolomics deals with the identification of all the metabolites and their interactions in a sample at any given time (Szewczyk and Kowalski 2016b). Since various enzymes participate in metabolic processes inside the microbes, inside the plant systems and also link these interactions as endophytic systems, therefore the integration of metabolomics data is essential. A few open-source tools or software that are used to study the metabolome include: MetaboLights (Haug et al. 2013), MetaboloAnalyst (Xia et al. 2015), OpenMS (Sturm et al. 2008) etc. Likewise, interactome is the domain which deals with the interaction networks.

Phenomics involves high-throughput analysis of the phenotype, which is determined by the genotype under the influence its environment. Unlike genomics which involves sequencing the whole genome, phenomics only involves the characterization of a particular set of phenotype (Deshmukh et al. 2016b). Technological advances such as automated image processing allows precise and speedy phenotyping. Similarly, Ionomics deals with the study of elemental composition involving high-throughput identification and quantification. Ionomics can be integrated with genomics to identify existing genetic differences and explore tolerance to stresses.

To conclude, genetically engineered microbes with increased potential for contaminant uptake and tolerance have undoubtedly unlocked new dimensions in bioremediation. Notably, omics techniques are in progressive phase and multifaceted connections need to be explored. Only one

omic technique might not be sufficient to understand the interactions in the microbial world, between microbes and the plant systems and between the dynamic environment and thus, an integrated approach is the need of the hour. Better understanding of processes involved in rhizosphere bioremediation essentially needs the researchers to look at the same biological question from various different perspectives and by using multiple omic strategies. The research in this domain is beginning to catch the pace and offers very promising growth potential. But at the same time molecular biologists need to address environmental and public health concerns. With the use of genetically engineered microbes in field conditions, bio-safety also needs to be assessed. More advancement in the understanding of microbe assisted bioremediation could prove to be very beneficial in the biotechnological sector and can revolutionize the fight against global food security challenge threatened by human activities that degrade the environment.

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Steady Magnetohydrodynamic Micropolar Fluid Flow and Heat and Mass Transfer in Permeable Channel with Thermal Radiation

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Abstract: The present work is devoted to the study of magnetohydrodynamic micropolar fluid flow in a permeable channel with thermal radiation. The Rosseland approximation for thermal radiation is taken into account in the modelling of heat transfer. The governing equations are expressed in non-dimensional form. The Homotopy Perturbation Method (HPM) is briefly introduced and applied to derive the solution of nonlinear equations. The effects of various involved parameters like Reynolds number, microrotation parameter and Prandtl number on flow and heat transfer are discussed. Further, their effects on Nusselt and Sherwood numbers are also investigated from the physical point of view. Analytic solutions of the problem are obtained by HPM and a numerical technique bvp4c package MATLAB is applied to predict the graphs between different parameters.

Keywords: micropolar fluid; permeable channel; homotopy perturbation method; heat transfer; magnetohydrodynamic; thermal radiation.

1. Introduction

From the industrial point of view, the process of heat and mass transfer has a great impact. Many researchers concentrate on this area. In particular, in the metallurgical industry, an application of heat transfer criterion has been studied with magnetohydrodynamic micropolar fluid flows. Mohamed and Abo-Dahab [1], Perdikis and Raptis [2] and Raptis [3] discussed the impact of heat and mass transfer in micropolar and magnetohydrodynamic micropolar fluid flows in the presence of various characteristics like thermal radiation, heat generation, and porous media. Seddeek et al. [4] obtained the analytic solution of the problem leading to the effect of radiation on the flow of a magneto-micropolar fluid past a continuously moving plate with suction and blowing. El-Arabawy [5] observed the behaviour of suction and injection in his problem. On the other hand, Sharma and Gupta [6] studied the effects of porosity and thermal convection on micropolar fluids. The numerical simulation of the solution of micropolar fluid flows with suction and injection has been discussed by Subhadra et al. [7], Takhar et al. [8], Kelson and Farrell [9], and Muhammad et al. [10]. A few years ago, the flows in permeable channels and circular pipes made a considerable impact, drawing attention to these researchers.

In his work, Berman [11] showed that the mathematical equations could be reduced to a single 4th-order nonlinear ordinary differential. Terrill and Shrestha [12] and Asghar et al. [13] studied the behaviour of permeabilities in channel flows. Most of the scientific problems are nonlinear in nature, and such problems do not have an analytic solution; therefore, other methods like ADM (Adomian decomposition method) and HPM (homotopy perturbation method) can be applied to obtain an analytic solution to these types of scientific problems. In recent years, due to its simplicity and growing interest, the homotopy perturbation technique in nonlinear problems has been applied. Berman [14,15] developed and formulated the homotopy perturbation method and proved that this method is compatible with nonlinear physical problems. Ganji [16], Biazar [17], Fereidoon et al. [18], Hemeda [19], Aminikhah and Hemmatnezhad [20], Soltanian et al. [21], and Yildirim [22] have applied HPM to obtain analytic solutions. Sheikholeslami [23,24] also used the HPM method in his studies. Heat transfer in a permeable channel in the presence of micropolar fluid flow using the analytic method was investigated by M. Sheikholeslami and M. Hatami and D.D. Ganji [25], and A. Mirzaaghaian and D.D. Ganji [26] found the DTM solution for micropolar fluid flow and heat transfer through permeable walls. Homotopy perturbation technique has been used by J.H. He [27]. P. Sibanda et al. [28] observed the flow of a micropolar fluid in channel with heat and mass transfer and H. Mirgolbabaei et al. [29] studied semi-analytic investigation on micropolar fluid flow and heat transfer in a permeable channel using AGM. Some of the boundary value problems of micropolar fluid flow were investigated by Bhupander Singh [30–32]. Hayder I. Mohammed et al. [33] investigated the improved melting of latent heat storage via a porous medium and uniform Joule heat generation, and Milad Ghaneifar et al. [34] analyzed hybrid nanofluid flow and the heat transfer characteristics of a heat sink partially fitted with a multilayered porous medium. Mohammad Ghalambaz et al. [35] addressed the melting flow and heat transfer of electric conductive phase change materials (PCMs) subject to a variable magnetic field in a cavity enclosure and determined that the effect of the magnetic field on the melting behavior of PCM is negligible at the initial stages of melting. H. Ali Farooq et al. [36] studied numerically the MHD mixed convection due to a rotating circular solid cylinder in a trapezoidal enclosure filled with Cu-water nanofluid saturated with a porous media, and found that the vertical magnetic field decreased stream function values more than inclined and horizontal fields. M. Ghalambaz et al. [37] investigated the flow and thermal behavior of nano-encapsulated phase change materials (NEPCM) dispersed in a liquid over a vertical flat plate, and found that the decrease in fusion temperature of NEPCM cores enhances heat transfer.

In the present paper, we applied HPM to find the approximate series solutions of velocity, microrotation, temperature, and solute concentration; their graphical representations were obtained by `bvp4c` routine in MATLAB to observe how velocity, micro-elements, temperature, and mass concentration are influenced by Reynolds numbers, Prandtl number, micropolar parameter, spin gradient viscosity, and Peclet number for the diffusion of heat and mass.

2. Formulation of the Problem

Here, a laminar incompressible micropolar fluid was considered along a two-dimensional permeable channel with porous walls through which fluid was uniformly injected or removed with velocity v_0 . The walls of the channel were taken to be parallel to x -axis at $2h$ distance apart with lower boundary $y = -h$; upper $y = h$; and y -axis taken perpendicular to the walls. The lower channel wall was maintained at temperature T_1 and solute concentration C_1 , while the upper wall was maintained with temperature T_2 and solute concentration C_2 . A uniform magnetic field B was applied perpendicular to the channel walls. (Figure 1).

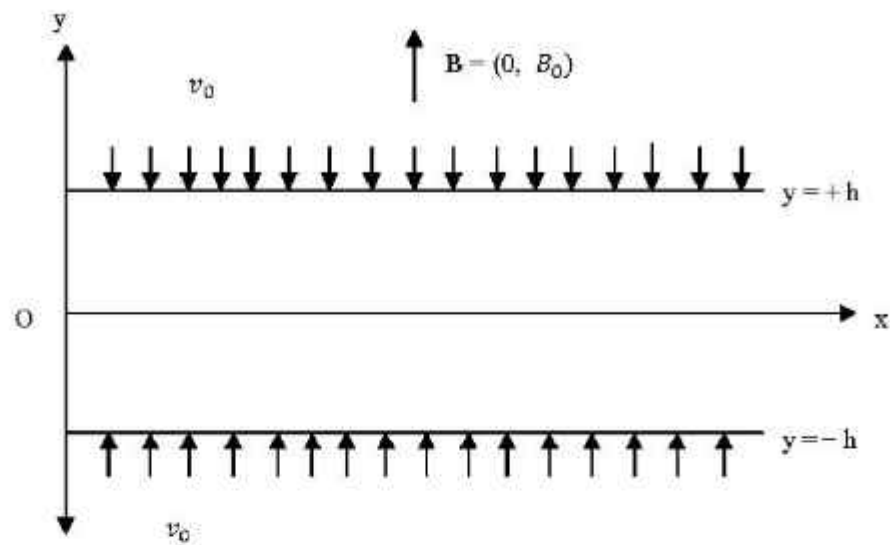


Figure 1. Geometrical view of the problem.

Following [25,26], the governing equations of the problem are

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0 \quad (1)$$

$$\rho \left(u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} \right) = -\frac{\partial P}{\partial x} + (\mu + \kappa) \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right) + \kappa \frac{\partial N}{\partial y} - \sigma B_0^2 u \quad (2)$$

$$\rho \left(u \frac{\partial N}{\partial x} + v \frac{\partial N}{\partial y} \right) = -\frac{\kappa}{j} \left(2N + \frac{\partial u}{\partial y} - \frac{\partial v}{\partial x} \right) + \frac{v_s}{j} \left(\frac{\partial^2 N}{\partial x^2} + \frac{\partial^2 N}{\partial y^2} \right) \quad (3)$$

$$\rho \left(u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} \right) = -\frac{\partial P}{\partial y} + (\mu + \kappa) \left(\frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} \right) - \kappa \frac{\partial N}{\partial x} \quad (4)$$

$$\rho \left(u \frac{\partial T}{\partial x} + v \frac{\partial T}{\partial y} \right) = \frac{K_1}{C_p} \frac{\partial^2 T}{\partial y^2} - \frac{1}{C_p} \frac{\partial q_r}{\partial y} \quad (5)$$

$$\rho \left(u \frac{\partial C}{\partial x} + v \frac{\partial C}{\partial y} \right) = D^* \frac{\partial^2 C}{\partial y^2} \quad (6)$$

where u and v denote the velocity components along x -axis and y -axis directions, respectively, and ρ is the fluid density, μ is the dynamic viscosity, κ is the material parameter, N is the microrotation velocity, P is the fluid pressure and T , C and C_p are the fluid temperature, species concentration and specific heat at constant pressure, respectively, K_1 is the thermal conductivity, D^* is the molecular diffusivity, j is the micro-inertia density, $v_s = (\mu + \frac{\kappa}{2})j$ is the microrotation viscosity and q_r is the radiative heat flux.

The appropriate boundary conditions are

$$u = 0, v = -v_0, N = -s \frac{\partial u}{\partial y}, T = T_1, C = C_1 \text{ at } y = -h \quad (7)$$

$$u = \frac{u_0 x}{h}, v = v_0, N = -s \frac{\partial u}{\partial y}, T_1 = T_2, C = C_2 \text{ at } y = +h \quad (8)$$

where s denotes the boundary parameter representing the degree to which microelements are free to rotate near the channel walls. The case $s = 0$ means the strong concentration which shows that microelements are not rotating near the channel walls, $s = 1/2$ shows the weak concentrations and vanishing of the anti-symmetric part of the stress tensor, whereas

$s = 1$ represents turbulent flow [26]. We introduced the following non-dimensional similarity variables in the case of strong concentration as:

$$\eta = \frac{y}{h}, \psi = -v_0 x f(\eta), N = \frac{v_0 x}{h^2} g(\eta), \theta(\eta) = \frac{T - T_1}{T_2 - T_1}, \phi(\eta) = \frac{C - C_1}{C_2 - C_1} \quad (9)$$

where, $T_2 = T_1 + Ax$, $C_2 = C_1 + Bx$ with A and B as constants [26]. The velocity components of flow are defined by stream function as $u = \frac{\partial \psi}{\partial y}$, $v = -\frac{\partial \psi}{\partial x}$.

Using Similarity Transformations (9), Equations (2)–(4), after eliminating, p become

$$(1 + N_1)f'''' - N_1g'' - Re(f'f'' - ff''') - Mf'' = 0 \quad (10)$$

$$N_2g'' + N_1(f'' - 2g) - N_3Re(fg' - f'g) = 0 \quad (11)$$

Using Roseland approximation, we have $q_r = -\frac{4\sigma_0}{3k_0} \frac{\partial T^4}{\partial y}$ where σ_0 the Stefan Boltzmann constant is and k_0 is the mean absorption coefficient. Assuming that the difference in temperature within the flow is such that T^4 can be expressed as linear combination of the temperature. Therefore expanding T^4 about T_∞ and ignoring higher order terms, we have $T^4 \cong -3T_\infty^4 + 4T_\infty^3 T$

$$q_r = -\frac{4\sigma_0}{3k_0} \frac{\partial}{\partial y} (-3T_\infty^4 + 4T_\infty^3 T) = -\frac{16\sigma_0 T_\infty^3}{3k_0} \frac{\partial T}{\partial y} = -\frac{16\sigma_0 T_\infty^3}{3k_0} \left(\frac{Ax}{h^2} \theta'(\eta) \right) \quad (12)$$

Using Similarity Transformations (9) and Equation (12), Equations (5) and (6) lead to

$$\theta'' + \frac{Peh}{\left(1 + \frac{4}{3}Nr\right)} (f'\theta - f\theta') = 0 \quad (13)$$

$$\phi'' + Pem(f'\phi - f\phi') = 0 \quad (14)$$

With Boundary Conditions

$$f'(-1) = 0, f(-1) = -1, g(-1) = 0, \theta(-1) = 0, \phi(-1) = 0 \quad (15)$$

$$f'(1) = -1, f(1) = 1, g(1) = 1, \theta(1) = 1, \phi(1) = 1 \quad (16)$$

where $N_1 = \frac{\kappa}{\mu}$ is the micro-polar parameter, $M = \frac{\sigma B_0^2 h^2}{\mu}$ is the Magnetic parameter, $N_2 = \frac{v_0}{\mu h^2}$ is the spin gradient viscosity parameter, $N_3 = \frac{j}{h^2}$, $Nr = \frac{4\sigma_0 T_\infty^3}{\kappa_1 k_0}$ is the Radiation parameter, $Pr = \frac{v \rho C_p}{\kappa_1}$ is the Prandtl number. $Re = \frac{v_0 h}{\nu}$ is the Reynolds number [for suction $Re > 0$, for injection $Re < 0$], $Peh = Pr Re$ is the Peclet number for diffusion of heat, $Pem = Sc Re$ is the Peclet number for diffusion of mass and $Sc = \frac{\nu}{D^*}$ is the generalized Schmidt number.

The physical quantities which are of greatest interest are local Sherwood number and local Nusselt number which are defined as follows:

$$S_{hx} = -\vartheta'(-1) \quad (17)$$

$$N_{ux} = -\left(1 + \frac{4Nr}{3}\right) \theta'(-1) \quad (18)$$

3. Analysis of the Homotopy Perturbation Method (HPM)

The basic idea of HPM is illustrated in [27]. Accordingly, we consider the following equation

$$A(u) - f(r) = 0, r \in \Omega \quad (19)$$

with the boundary condition

$$B\left(u, \frac{\partial u}{\partial n}\right) = 0, r \in \Gamma, \quad (20)$$

where A is a general differential operator, B a boundary operator, $f(r)$ a known analytical function and Γ is the boundary of the domain Ω . According to the method, A is divided into two parts which are L and N , where, L is linear operator and N is nonlinear. Therefore, the Equation (19) can be rewritten as follows:

$$L(u) + N(u) - f(r) = 0, r \in \Omega. \tag{21}$$

Now the homotopy perturbation structure is shown as follows:

$$H(v, p) - (1 - p)[L(v) - L(u_0)] + p[A(v) - f(r)] = 0 \tag{22}$$

where,

$$v(r, p) : \Omega \times [0, 1] \rightarrow R. \tag{23}$$

In the Equation (22), $p \in [0, 1]$ is an embedding parameter and u_0 is the first approximation that satisfies the boundary condition. We can assume that the solution of the Equation (23) can be written as a power series in p , as follows:

$$v = v_0 + pv_1 + p^2v_2 + \dots \tag{24}$$

and the best approximation for solution is given as

$$u = \lim_{p \rightarrow 1} v = v_0 + v_1 + v_2 + \dots \tag{25}$$

4. Solution of the Problem by HPM

In order to solve the nonlinear differential Equations (10), (11), (13) and (14) by HPM, we constructed a homotopy as follows:

$$H(f, p) = (1 - p)(f^{iv} - f_0^{iv}) + p[(1 + N_1)f^{iv} - N_1g'' - R_c(ff'' - f'f') - Mf'] = 0 \tag{26}$$

$$H(g, p) = (1 - p)(g'' - g_0'') + p[N_2g'' + N_1(f'' - 2g) - N_3R_c(fg' - f'g)] = 0 \tag{27}$$

$$H(\theta, p) = (1 - p)(\theta'' - \theta_0'') + p\left(1 + \frac{4}{3}N_r\right)\theta'' + p[P_{ch}(f'\theta - f\theta')] = 0 \tag{28}$$

$$H(\phi, p) = (1 - p)(\phi'' - \phi_0'') + p[\phi'' + P_{cm}(f'\phi - f\phi')] = 0 \tag{29}$$

Now f , g , θ and ϕ can be explained as follows:

$$f = f_0 + pf_1 + p^2f_2 + \dots \tag{30}$$

$$g = g_0 + pg_1 + p^2g_2 + \dots \tag{31}$$

$$\theta = \theta_0 + p\theta_1 + p^2\theta_2 + \dots \tag{32}$$

$$\phi = \phi_0 + p\phi_1 + p^2\phi_2 + \dots \tag{33}$$

Substituting Equations (30)–(33) into Equations (26)–(29) and simplifying and re-arranging based on power of p -terms, we obtain:

for p^0 :

$$f_0^{iv} = 0, g_0'' = 0, \theta_0'' = 0, \phi_0'' = 0 \tag{34}$$

and boundary conditions are:

$$\left. \begin{aligned} \eta = -1 : f_0 = -1, f_0' = g_0 = \theta_0 = \phi_0 = 0 \\ \eta = +1 : f_0 = g_0 = \theta_0 = \phi_0 = 1, f_0' = -1 \end{aligned} \right\} \tag{35}$$

for p^1 :

$$\left. \begin{aligned} (1 + N_1)f_1^{iv} - N_1g_0'' - R_c(f_0f_0'' - f_0'f_0'') - Mf_0' &= 0 \\ N_2g_1'' + N_1(f_0'' - 2g_0) - N_3R_c(f_0g_0' - f_0'g_0) &= 0 \\ \left(1 + \frac{4}{3}N_r\right)\theta_1'' + P_{ch}(f_0'\theta_0 - f_0\theta_0') &= 0 \\ \phi_1'' + P_{em}(f_0'\phi_0 - f_0\phi_0') &= 0 \end{aligned} \right\} \quad (36)$$

and boundary conditions are:

$$\left. \begin{aligned} \eta = -1 : f_1 = f_1' = g_1 = \theta_1 = \phi_1 = 0 \\ \eta = +1 : f_1 = g_1 = \theta_1 = \phi_1 = f_1' = 0 \end{aligned} \right\} \quad (37)$$

Solving Equations (34) and (36) with boundary conditions, we have

$$\left. \begin{aligned} f_0(\eta) &= -0.75\eta^3 - 0.25\eta^2 + 1.75\eta + 0.25 \\ g_0 &= 0.5\eta + 0.5 \\ \theta_0 &= 0.5\eta + 0.5 \\ \phi_0 &= 0.5\eta + 0.5 \end{aligned} \right\} \quad (38)$$

$$\begin{aligned} f_1(\eta) &= -\frac{R_c}{1+N_1}[0.0080357143]\eta^7 - 0.00625\left[\frac{R_c+M}{1+N_1}\right]\eta^6 \\ &- \left[\frac{R_c}{1+N_1}(0.0020833333) + \frac{M}{1+N_1}(0.0041666667)\right]\eta^5 \\ &+ \left[\frac{R_c}{1+N_1}(0.0260416667) + \frac{M}{1+N_1}(0.0729166667)\right]\eta^4 \\ &+ \left[\frac{R_c}{1+N_1}(0.360863095) - \frac{M}{1+N_1}(0.089453125)\right]\eta^3 \\ &+ \left[\frac{R_c}{1+N_1}(0.360863095) - \frac{M}{1+N_1}(0.089453125)\right]\eta^2 \\ &- \left[\frac{R_c}{1+N_1}(0.03333333334) + \frac{M}{1+N_1}(0.209375)\right]\eta^2 \\ &\quad + \frac{M}{1+N_1}(0.124609375)\eta \\ &+ \left[\frac{M}{1+N_1}(0.11171875) - \frac{R_c}{1+N_1}(0.041592261)\right] \end{aligned} \quad (39)$$

$$\begin{aligned} g_1(\eta) &= \frac{N_3R_c}{N_2}(0.375)\eta^5 + \frac{N_1R_c}{N_2}(0.1041666667)\eta^4 \\ &+ \left[\frac{N_3R_c}{N_2}(0.0416666667) + \frac{N_1}{N_2}(0.9166666667)\right]\eta^3 \\ &\quad + \left[\frac{N_1}{N_2}(0.75) - \frac{N_3R_c}{N_2}(0.375)\right]\eta^2 \\ &- \left[\frac{N_3R_c}{N_2}(0.0761666667) + \frac{N_1}{N_2}(0.9166666667)\right]\eta \\ &\quad + \left[\frac{N_1R_c}{N_2}(0.2708333333) - \frac{N_1}{N_2}(0.75)\right] \end{aligned} \quad (40)$$

$$\begin{aligned} \theta_1(\eta) &= \frac{P_{ch}}{1+\frac{4}{3}N_r}[0.0375\eta^5 + 0.1041666667\eta^4 + 0.0416666667\eta^3 - 0.375\eta^2] \\ &- \left[\frac{P_{ch}}{1+\frac{4}{3}N_r}(0.0791666667)\right]\eta + \left[\frac{P_{ch}}{1+\frac{4}{3}N_r}(0.2708333333)\right] \end{aligned} \quad (41)$$

$$\begin{aligned} \phi_1(\eta) &= P_{em}[0.0375\eta^5 + 0.1041666667\eta^4 + 0.0416666667\eta^3 - 0.375\eta^2] \\ &- P_{em}(0.0791666667)\eta + P_{em}(0.2708333333) \end{aligned} \quad (42)$$

The terms $f_i(\eta), g_i(\eta), \theta_i(\eta)$ and $\phi_i(\eta)$ for $i \geq 2$ are too large to present graphically. The solution of the Equations (30)–(33), where $p = 1$ will be as

$$\left. \begin{aligned} f(\eta) &= f_0(\eta) + f_1(\eta) + f_2(\eta) + \dots \\ g(\eta) &= g_0(\eta) + g_1(\eta) + g_2(\eta) + \dots \\ \theta(\eta) &= \theta_0(\eta) + \theta_1(\eta) + \theta_2(\eta) + \dots \\ \phi(\eta) &= \phi_0(\eta) + \phi_1(\eta) + \phi_2(\eta) + \dots \end{aligned} \right\} \quad (43)$$

5. Results and Discussions

The transformed Equations (10), (11), (13) and (14) along with the boundary conditions (15) and (16) were solved numerically by the bvp4c routine in MATLAB and the results thus obtained were developed into graphs in which the behaviour of non-dimensional parameters like N_1 , N_2 , N_3 , Re , Peh , Pem etc. on the simulated fluid velocity, microrotation profile and temperature profiles are shown through Figures 2–13.

Figures 2–4 depicts the effect of N_1 , N_2 and N_3 respectively on simulated velocity. As in Figure 2 when $\eta < -0.4545$ velocity decreases and after that it start increasing and as $\eta > 0.4949$ it again decreases. That is, N_1 has a dual effect on simulated velocity while on increasing N_2 and N_3 , the simulated velocity decreases (see Figures 3 and 4).

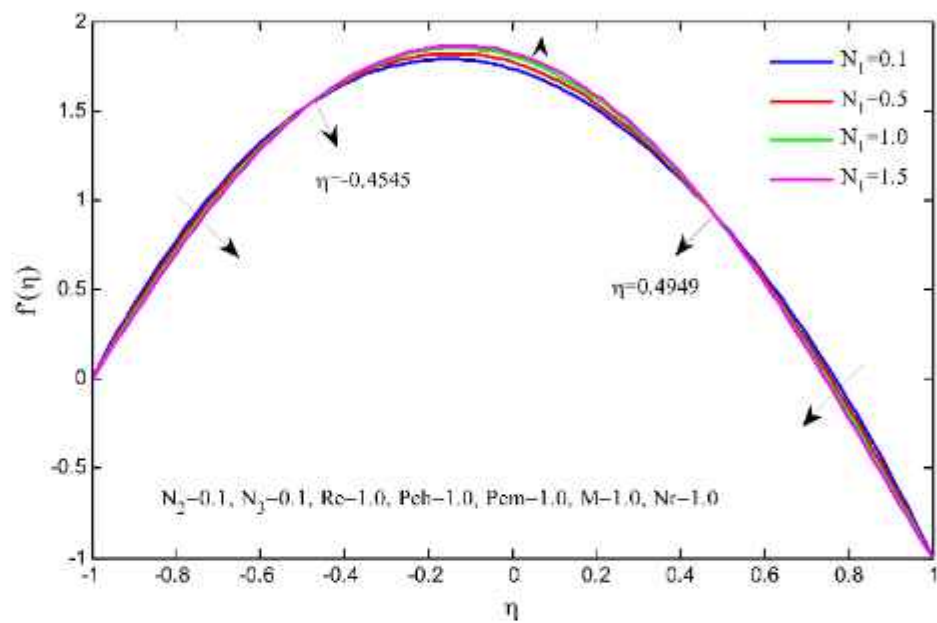


Figure 2. Effect of N_1 on simulated velocity.

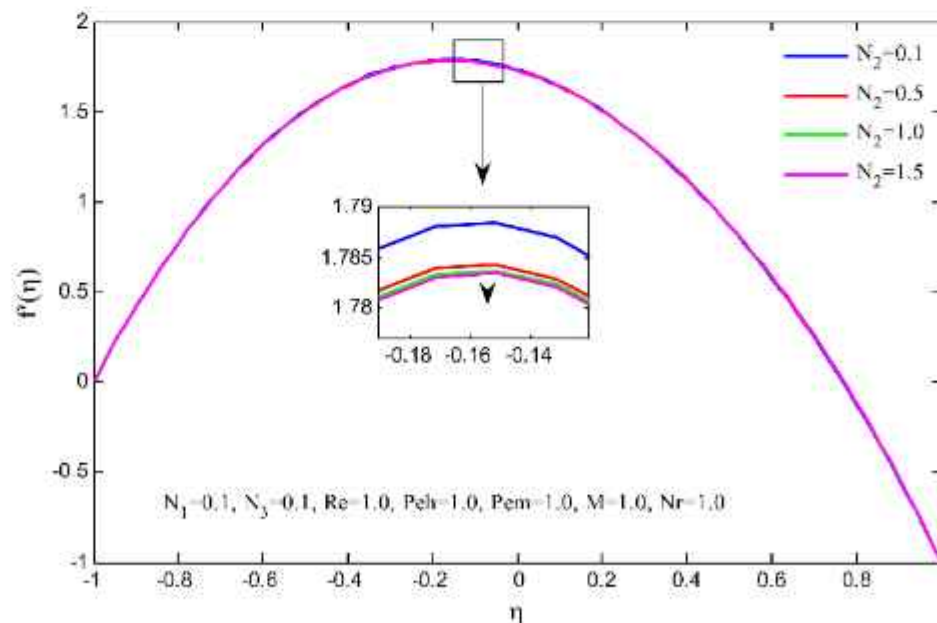


Figure 3. Effect of N_2 on simulated velocity.

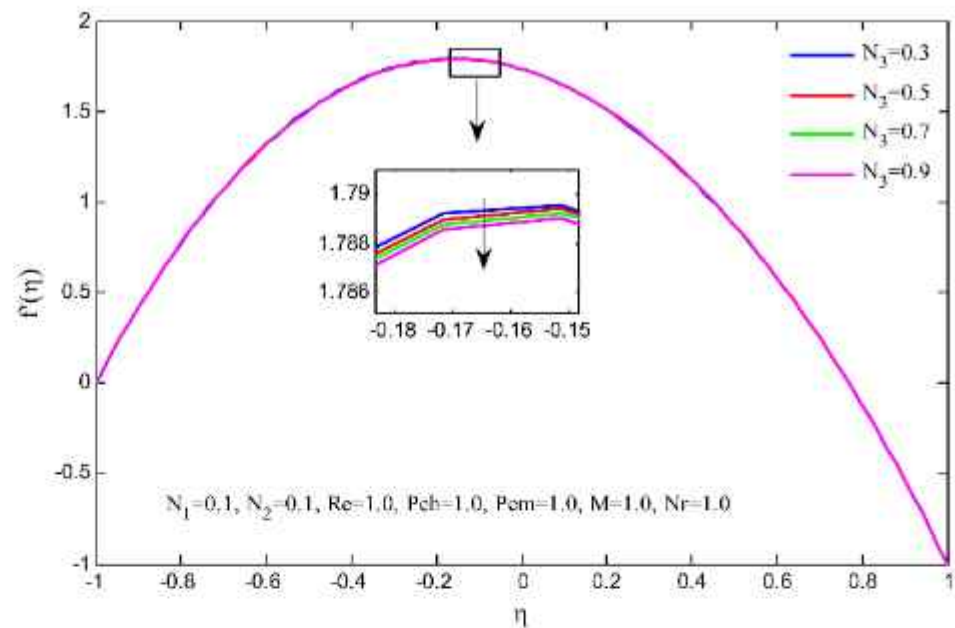


Figure 4. Effect of N_3 on simulated velocity.

It can be seen in Figure 5 that keeping the other parameters fixed, when $\eta < -0.6566$ velocity decreases for $Re < 2.0$ and an increase in velocity is shown for $Re > 2.0$, it starts decreasing when $-0.6566 < \eta < 0.3535$ and it start increasing when $\eta > 0.3535$. As the Reynolds number increases, the fluid velocity decreases in the middle of the channel, whereas near the lower wall of the channel it decreases when the Reynolds number remains below 2.0; it starts increasing when the Reynolds number is above 2.0, but near the upper wall of the channel, it appears to increase when the Reynolds number increases from 1.0 to 4.0.

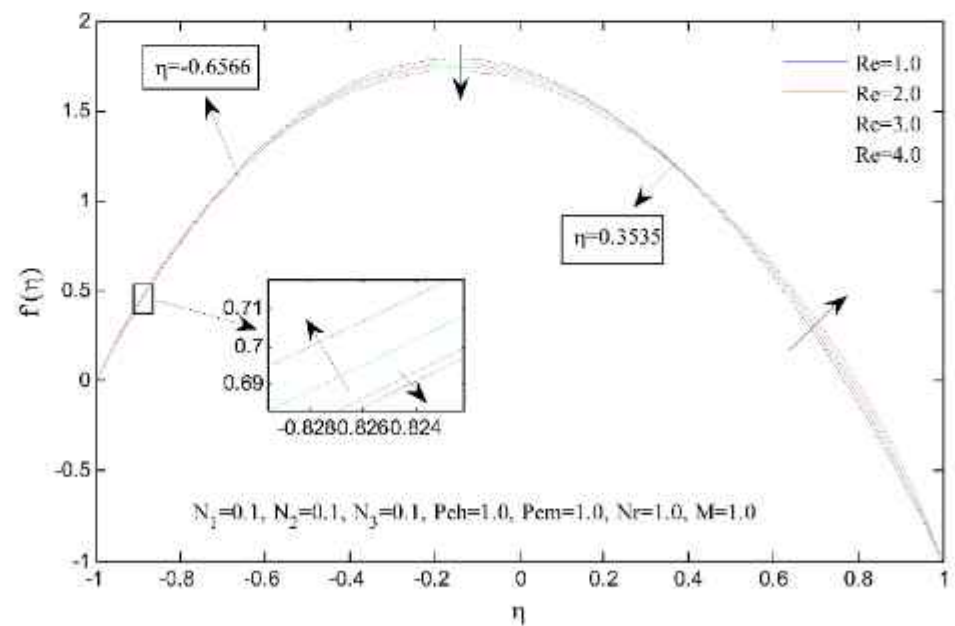


Figure 5. Effect of Re on simulated velocity.

Figure 6 portrays the effect of the magnetic field on simulated velocity. It increases as M increases, but after reaching $\eta = -0.05051$, the velocity decreases. In the presence of the magnetic field, the fluid oscillates irregularly in the middle of the channel.

Figure 7 shows that the micro-elements of the fluid irregularly oscillate around the middle of the channel as N_1 increases. As the coupling parameter gradually increases, it is reflected by the irregular oscillation of micro-elements around the middle of the channel.

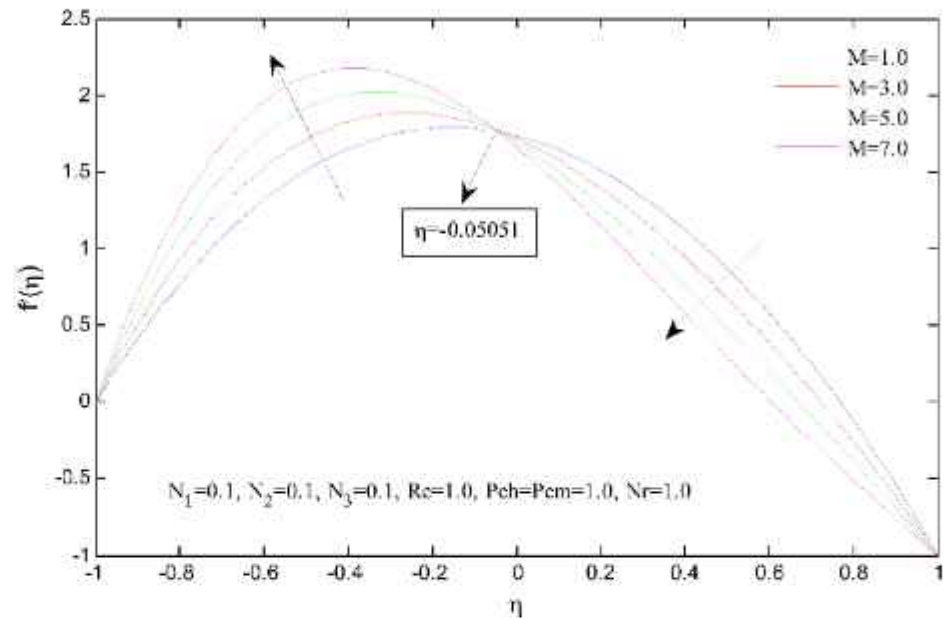


Figure 6. Effect of M on simulated velocity.

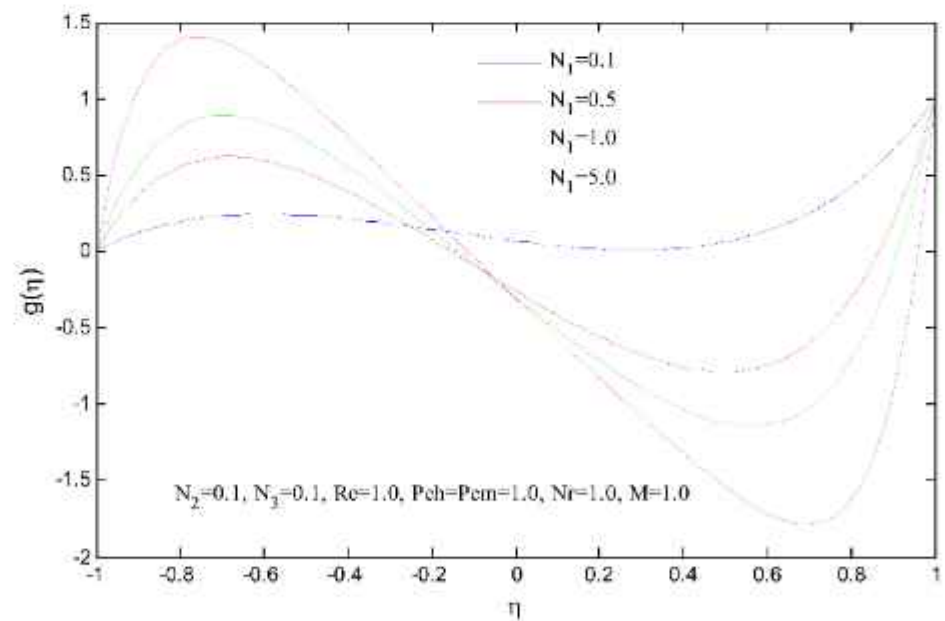


Figure 7. Effect of N_1 on microrotation profile.

Figure 8 shows that with an increase in N_2 , micro-elements irregularly oscillate around $\eta < -0.5$, that is, due to the increase of spin gradient viscosity, micro-elements oscillate in an irregular mode near the lower wall of the channel.

An increase in N_3 leads to the irregular oscillation of micro-elements around $\eta < -0.3$, that is, the micro-elements oscillate near the lower wall of the channel as N_3 increases (Figure 9).

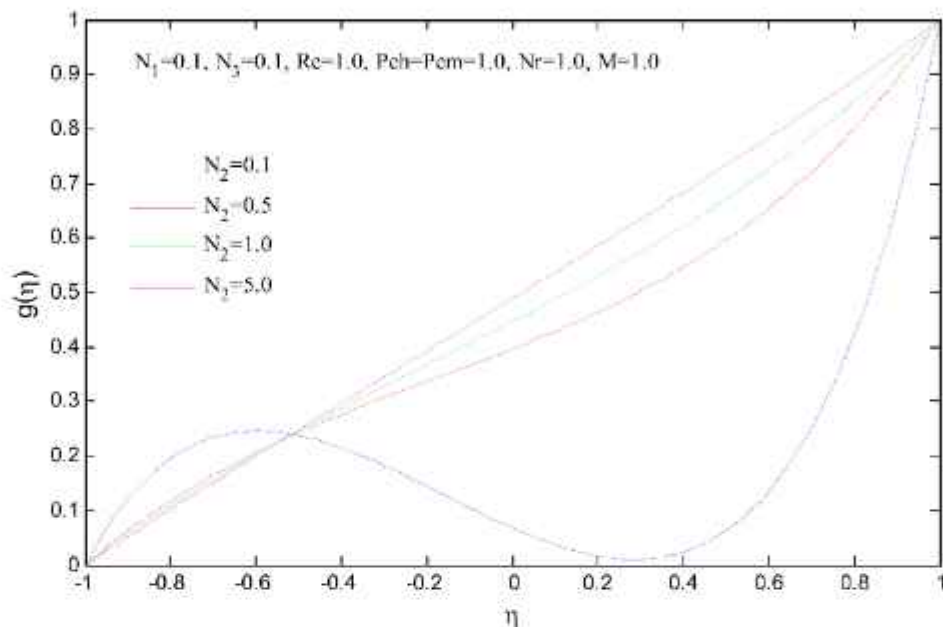


Figure 8. Effect of N_2 on microrotation profile.

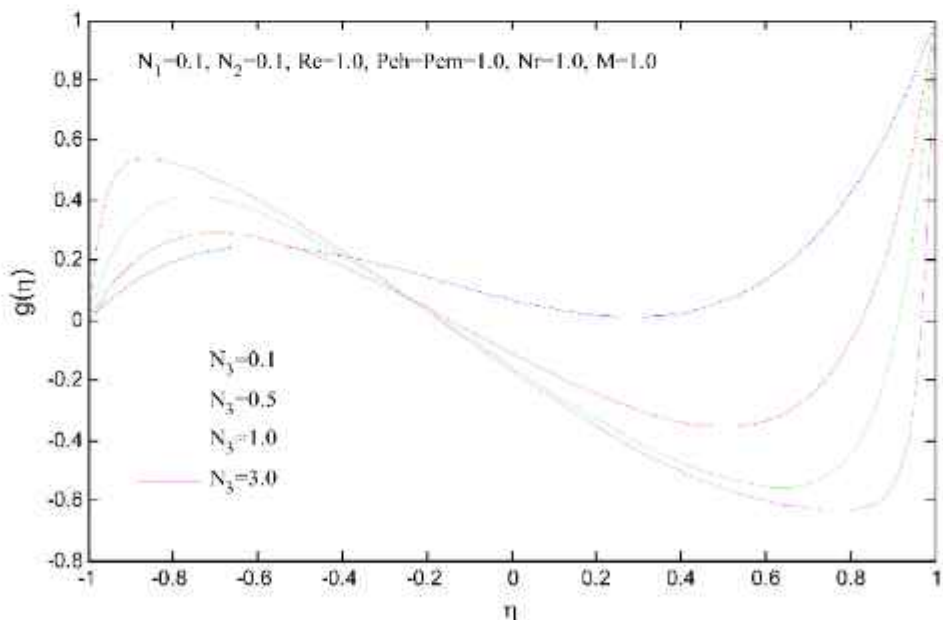


Figure 9. Effect of N_3 on microrotation profile.

Figure 10 demonstrates that the micro-elements irregularly oscillate near the lower wall of the channel at around $(-0.6, -0.4)$ as Reynolds number increases.

Figure 11 shows that the increase in the value of N_r leads to a decrease in the temperature field and that the maximum decrease in temperature is at the middle of the channel, indicating that larger the radiation parameter lower the temperature in the middle of the channel.

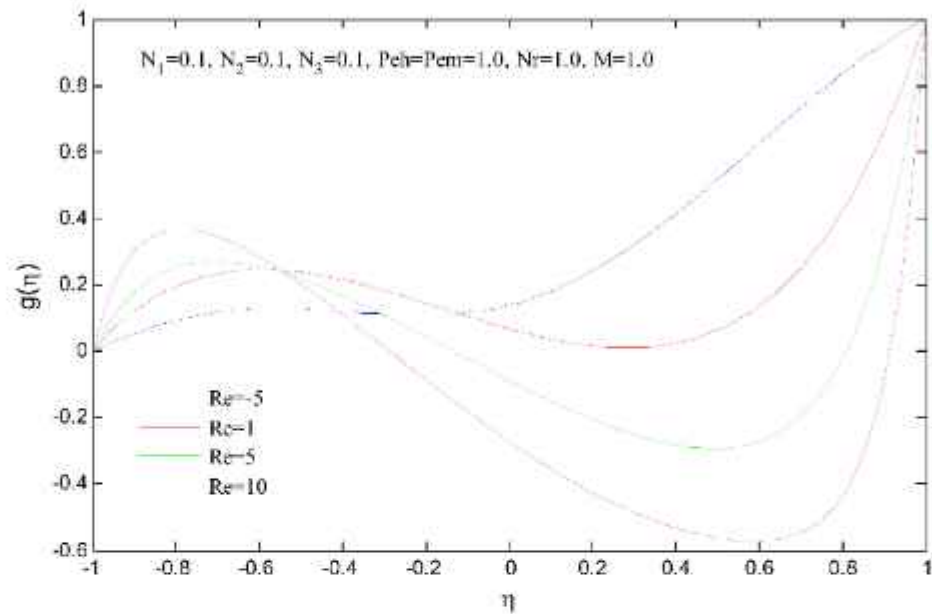


Figure 10. Effect of Re on microrotation profile.

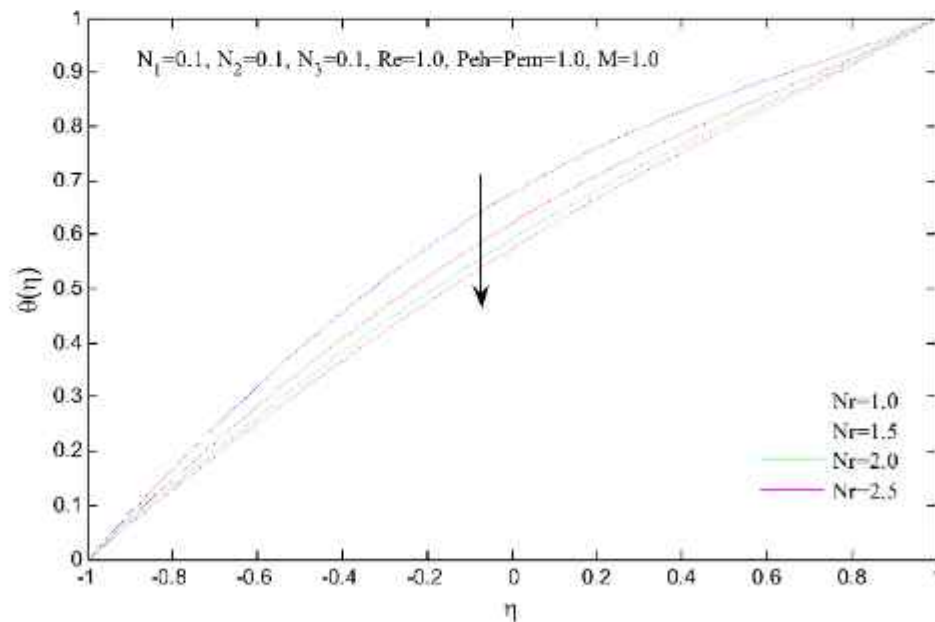


Figure 11. Effect of N_r on temperature profile.

In Figure 12, it can be observed that the fluid temperature increases in the middle of the channel when $Peh = 0.5$ to $Peh = 1.5$, and the temperature rapidly increases when $Peh = 1.5$ onwards.

Figure 13 shows the effect of Peclet number for the diffusion of mass in a concentration profile. The solute concentration will be at a maximum in the middle of the channel as the diffusion of mass increases but it remains non-zero at the upper wall of the channel.

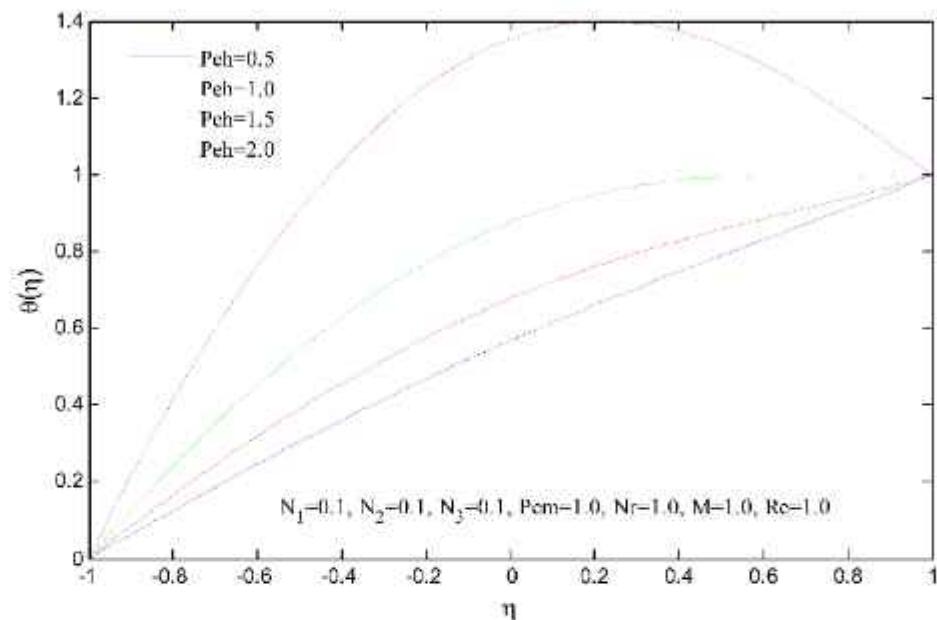


Figure 12. Effect of P_{eh} on temperature profile.

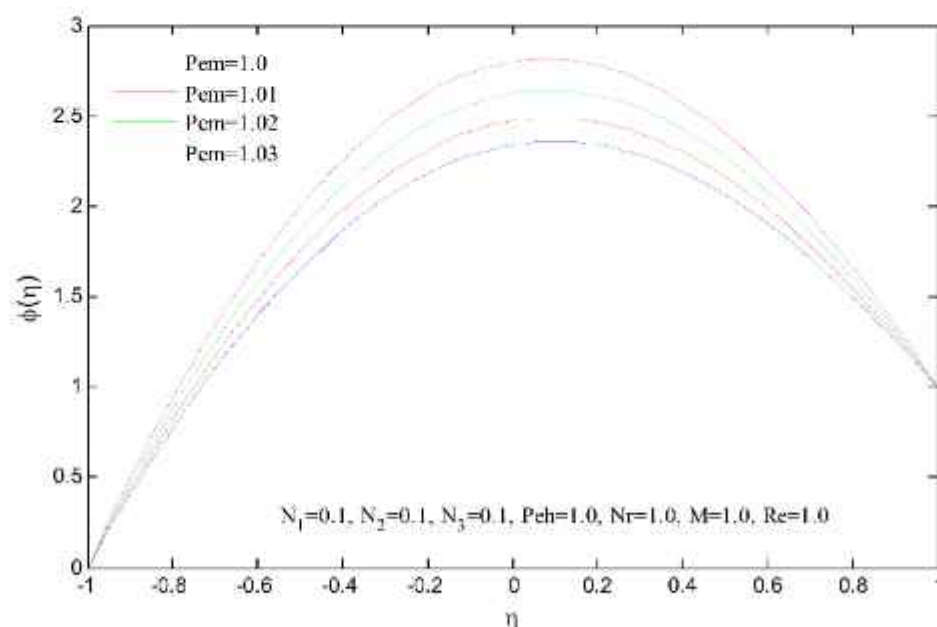


Figure 13. Effect of P_{em} on mass concentration profile.

6. Conclusions

The micropolar parameter N_1 has a dual effect on simulated velocity while the spin gradient viscosity parameter N_2 and N_3 both have an adverse effect on simulated velocity.

On increasing N_1 , the microrotation irregularly oscillates around the middle of the channel, whereas on increasing spin gradient viscosity N_2 , micro-elements irregularly

oscillate around $\eta < -0.5$; an increase in N_3 leads to irregular oscillation at an angular velocity around $\eta < -0.3$.

The Reynolds number impacts dual effect on simulated velocity about $Re = 2.0$ when $-1 < \eta < -0.6566$ and increasing the Reynolds number leads to irregular oscillations in microrotation.

The simulated velocity irregularly oscillates around $\eta = -0.05051$, when M increases. N_r plays a key role in decreases in the temperature profile and the maximum fall in temperature is noticed near middle of the channel.

The fluid temperature increases in the middle of the channel when $Peh = 0.5$ to $Peh = 1.5$ and the temperature rapidly increases when $Peh = 1.5$ onwards; the upper wall of the channel will experience increases in temperature but the lower wall will have negligible increases in temperature.

Peclet number Pem for diffusion of mass indicates that mass diffusion will be at a maximum in the middle of the channel.

From Table 1, it has been observed that local heat flux increases when $M < 10$ and starts decreasing when $M > 10$, and inversely depends on Reynolds number. Its dependence on N_2 and N_3 is inverse, while on N_1 it is directly proportional. The heat flux increases when the values of Peh increase, whereas it decreases when increasing the values of radiation parameter N_r .

Table 1. Numerical values of $-\theta'(-1)$ and $-\varphi'(-1)$ for different values of $N_1, N_2, N_3, Re, M, P_{ch}, P_{em}$ and N_r .

N_1	N_2	N_3	Re	M	P_{ch}	P_{em}	N_r	$-\theta'(-1)$
1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.1	0.5535
1.0	1.0	1.0	1.0	3.0	0.1	0.1	0.1	0.5536
1.0	1.0	1.0	1.0	5.0	0.1	0.1	0.1	0.5537
1.0	1.0	1.0	1.0	10.0	0.1	0.1	0.1	0.5537
1.0	1.0	1.0	1.0	15.0	0.1	0.1	0.1	0.5536
1.0	1.0	1.0	1.0	20.0	0.1	0.1	0.1	0.5535
1.0	1.0	1.0	3.0	1.0	0.1	0.1	0.1	0.5552
1.0	1.0	1.0	5.0	1.0	0.1	0.1	0.1	0.5538
1.0	1.0	1.0	10.0	1.0	0.1	0.1	0.1	0.5532
1.0	1.0	1.5	1.0	1.0	0.1	0.1	0.1	0.5532
1.0	1.0	1.6	1.0	1.0	0.1	0.1	0.1	0.5530
1.0	1.0	2.0	1.0	1.0	0.1	0.1	0.1	0.5520
1.0	1.5	1.0	1.0	1.0	0.1	0.1	0.1	0.5533
1.0	1.6	1.0	1.0	1.0	0.1	0.1	0.1	0.5532
1.0	2.0	1.0	1.0	1.0	0.1	0.1	0.1	0.5530
1.5	1.0	1.0	1.0	1.0	0.1	0.1	0.1	0.5537
1.6	1.0	1.0	1.0	1.0	0.1	0.1	0.1	0.5538
2.0	1.0	1.0	1.0	1.0	0.1	0.1	0.1	0.5539
1.0	1.0	1.0	1.0	1.0	0.2	0.1	0.1	0.6167
1.0	1.0	1.0	1.0	1.0	0.3	0.1	0.1	0.6922
1.0	1.0	1.0	1.0	1.0	0.4	0.1	0.1	0.7842
1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.2	0.5475
1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.3	0.5427
1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.4	0.5387
N_1	N_2	N_3	Re	M	P_{ch}	P_{em}	N_r	$-\varphi'(-1)$
1.0	1.0	1.0	1.0	1.0	0.1	0.1	0.1	0.5613
1.0	1.0	1.0	1.0	1.0	0.1	0.2	0.1	0.6355
1.0	1.0	1.0	1.0	1.0	0.1	0.3	0.1	0.7268
1.0	1.0	1.0	1.0	1.0	0.1	0.4	0.1	0.8420

Table 1 also predicts that the mass flux depends only on Pem and it starts increasing upon increasing Pem .

7. Comparison of This Work with Other Work

In the absence of the magnetic field and thermal radiation, the Equations (10), (13) and (14) reduce equations obtained by M. Sheikholeslami et al. [24], but we have taken different boundary conditions in our paper. If we take the same boundary conditions as taken by [24], then the graphical representation of microrotation in our Figures 7–10 will be reduced to the same as is shown in [24]. In the absence of the magnetic field and thermal radiation, the governing equations of our problem have been reduced to the governing equations as in A. Mirzaaghaian et al. [25], and if we take the boundary conditions to be the same as in [25], the graphical representation of the temperature profile matches with Figures 11 and 12 in our paper. Thus, in the presence of the magnetic field and thermal radiation, we have obtained the new results presented in the Results and Discussion section of this study.

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Nomenclature

C	species concentration
D^*	molecular diffusivity
K_1	thermal conductivity
B_0	strength of constant applied magnetic field
f	dimensionless stream function
g	dimensionless microrotation
h	half width of channel
j	micro-inertia density
M	magnetic parameter
N	microrotation/angular velocity
$N_{1,2,3}$	dimensionless parameters
N_{ux}	Local Nusselt number
S_{ux}	Local Sherwood number
Sc	Schmidt number
P	pressure
Pr	Prandtl number
P_{th}	Peclet number for diffusion of heat
P_{em}	Peclet number for diffusion of mass

q_r	radiative heat flux
Re	Reynolds number
T	fluid temperature
N_r	radiation parameter
s	microrotation boundary condition
(u, v)	Cartesian velocity components
(x, y)	Cartesian coordinate components parallel & normal to channel axis, respectively
HPM	homotopy perturbation method

Greek Symbols

η	similarity variable
μ	dynamic viscosity
ρ	Fluid density
ψ	stream function
σ	electric conductivity
θ	dimensionless temperature
ϕ	dimensionless mass transfer parameter
κ	coupling coefficient
ν_s	microrotation/spin-gradient viscosity

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Numerical analysis of heat transfer in magnetohydrodynamic micropolar jeffery fluid flow through porous medium over a stretching sheet with thermal radiation

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Abstract

The present study investigates the micropolar Jeffrey fluid flow in the presence of magnetic field across a stretching surface through porous medium. Through suitable similarity transformations, the governing partial differential equations are transformed into nonlinear ordinary differential equations and been solved numerically using MATLAB software (built-in solver called bvp4c boundary value problem fourth order collocation method) which establish the numerical solutions for such transformed nonlinear ordinary differential equations. The numerical solution for fluid velocity, micro-rotation and temperature thus obtained are explained graphically in which the influence of various pertinent parameters like magnetic parameter, porosity parameter, spin gradient viscosity parameter, unsteadiness parameter, Jeffrey fluid parameter, etc., on velocity, micro-rotation and temperature profiles has been studied and discussed. The plotted results are discussed for flow and heat transfer characteristics.

Keywords Micropolar fluid · Jeffrey fluid · Porous medium · Magnetohydrodynamic · Thermal radiation · Heat transfer

Introduction

The concept of micropolar fluids was formulated by Eringen [1] which include micro-components that couple the macroscopic velocity field and the particles rotational motion. These fluids are made of hard particles which are suspended in a viscous medium. This theory can be used to explain the flow of colloidal fluids, liquid crystals, biological structures, etc. The investigation in porous medium has been started with simple Darcy model, and a good account

of related convection problems in porous medium is given by Vafai and Hadim [2]. Rate of cooling is very essential for manufacture of the products so that a controlled cooling system is required. As the phenomenon of thermal radiation is known, it became very easy to handle the high amount of heat generated in many industrial processes such as nuclear reactor, space vehicle, etc. Thermal radiation is a process in which the energy, in the form of electromagnetic radiation, is emitted by a heated surface in all directions and travels directly to its point of absorption at the speed of light. The total radiant heat energy emitted by a surface is proportional to the fourth power of its absolute temperature which produces heat gradient term in energy equation. The classical Navier Stokes equations are not suitable to characterize the flow behavior of non-Newtonian fluids. Thus, the different types of non-Newtonian models are suggested in the literature. Inferable from its application in the business and in the innovative world, the non-Newtonian fluid has pulled in the consideration of researchers over the most recent couple of years. Particularly in a few designing applications, the non-Newtonian liquids regularly float away from the conduct of Newtonian liquids. There is one subclass of non-Newtonian liquids known as Jeffrey fluid which is one of the rate type materials. It shows the linear viscoelastic effect

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of fluid which has many applications in polymer industries. There are many examples of Jeffrey fluid including dilute polymer solution. Jeffrey fluid is a generally less complex direct model utilizing time derivative instead of convected derivative and utilizing by most fluid models. This liquid model is equipped for depicting the qualities of retardation and relaxation times. There is little writing accessible for these sorts of fluids; some of them can be found in (Hayat and Mustafa [3], Hayat and Obaidat [4], Nadeem and Fang [5], Turkyilmazoglu and Pop [6] and Qasim [7]). Anyways the time-subordinate boundary layer flows have been barely examined. Mukhopadhyay [8] investigated the impact of thermal radiation on the unsteady mixed convection flow and heat transfer limited by a permeable stretching surface installed in a porous medium. The temperamental mixed convection stagnation-point flow toward a stretching surface has been numerically investigated by Devi et al. [9]. Andersson et al. [10] inspected the heat transfer qualities on the flow induced by an unsteady stretching sheet. The unsteady boundary layer rotating flow because of the stretching surface has been researched by Nazar et al. [11].

The MHD boundary layer flow over stretching sheet is encountered numerously in many industrial and engineering that the MHD flow of non-Newtonian fluids was first studied by Sarpkaya [12]. From that point forward, there is a plenitude of writing that examines the MHD flows of non-Newtonian fluids over stretching sheet, and some of them can be found in Andersson [13], Liao [14], Sajid and Asghar [15], Ashokkumar and Pravin [16]. Literature examined has discovered the issue of Jeffrey liquid along with the impacts of MHD in permeable medium that has been considered by Nallapu and Radhakrishnamacharya [17, 18] and also by Ahmad and Ishak [19]. A definite closeness answer for the dimensionless differential framework was acquired. Some similarity solutions have been acquired for a few different highlights like viscoelasticity, magneto hydrodynamics, porosity, heat and mass transfer [20–23]. In spite of different actual constitutions of non-Newtonian liquids, among all the Jeffrey liquid is one what constituents portraying the perceptible highlights of retardation and relaxation times. [24–28]. Omar Abu Arqub et al. [31–35] examined heat fluid flow and numerical simulation in different characteristics like time-fractional partial differential equations and Gordon types equations based on reproducing kernel algorithm. Some researchers are doing their work in the area of numerical heat transfer. Some of them are S. Nazari, R. Ellahi and

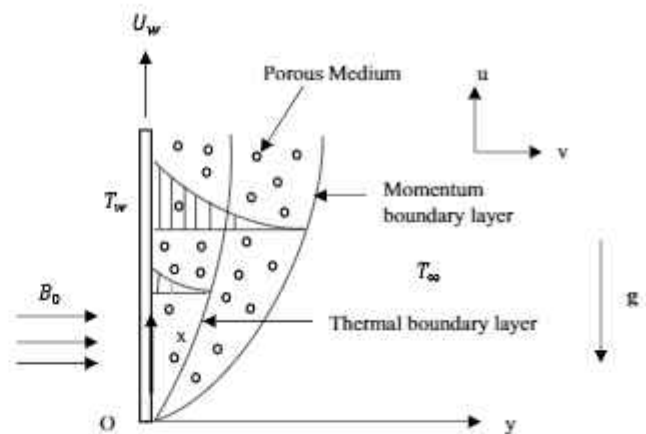


Figure 1 Geometry of the Problem

M.M. Sarafraz [36, 37] who numerically studied on mixed convection of non-Newtonian nanofluids in porous medium and thermal and flow characteristics of liquid flow in a 3D-printed micro-reactor. Before formation of our problem, we also review the literature of concern [38–48]. Persuaded to the importance and significant application toward Jeffrey liquid, the object of present investigation is to perform such examination for a micropolar Jeffrey liquid within the sight of thermal radiation passing over a stretching sheet in porous medium.

Mathematical formulation

An unsteady two-dimensional incompressible Jeffrey micropolar fluid flow in a porous medium over a stretching sheet coinciding with the plane $y = 0$ is considered, and the flow is confined to the plane $y > 0$. The surface is assumed to stretch linearly with velocity $U_w = ax$, where a is stretching constant. Here, the co-ordinates (x, y) is such that the x -axis is chosen parallel to the vertical surface, and the y -axis is taken normal to it, and (u, v) are the velocity components of the flow, and N defines the internal speed of the micropolar particles. g defines the gravity. A uniform magnetic field of strength B_0 is applied normal to the sheet. (Fig. 1.)

By invoking the boundary layer and Boussinesq approximations, the governing boundary layer equations [following T. Hayat, S. Asad, M. Mustafa and A. Alsaedi [24] and T. Hayat, Z. Iqbal, M. Mustafa and A. Alsaedi 29] for this problem can be written as

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0 \tag{1}$$

flow is such that T^4 can be expressed as linear combination of the temperature. Therefore expanding T^4 about T_∞ and ignoring higher order terms, we have $T^4 \cong -3T_\infty^4 + 4T_\infty^3 T$

$$\begin{aligned} \frac{\partial u}{\partial t} + u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} &= g\beta_T(T - T_\infty) - \frac{\sigma B_0^2 u}{\rho} + \left(\frac{K}{\rho}\right) \frac{\partial N}{\partial y} - \frac{\mu}{K * \rho} u \\ &+ \left(\frac{v + \frac{K}{\rho}}{1 + \lambda_2}\right) \left[\frac{\partial^2 u}{\partial y^2} + \lambda_1 \left(\frac{\partial^3 u}{\partial y^2 \partial t} + u \frac{\partial^3 u}{\partial x \partial y^2} + v \frac{\partial^3 u}{\partial y^3} - \frac{\partial u}{\partial x} \frac{\partial^2 u}{\partial y^2} + \frac{\partial u}{\partial y} \frac{\partial^2 u}{\partial x \partial y} \right) \right] \end{aligned} \tag{2}$$

$$\frac{\partial N}{\partial t} + u \frac{\partial N}{\partial x} + v \frac{\partial N}{\partial y} = \frac{\gamma}{\rho j} \frac{\partial^2 N}{\partial y^2} - \frac{\kappa}{\rho j} \left(2N + \frac{\partial u}{\partial y} \right) \tag{3} \quad \therefore \frac{\partial q_r}{\partial y} = -\frac{16\sigma_0 T_\infty^3}{3K_1} \frac{\partial^2 T}{\partial y^2} \tag{5}$$

$$\frac{\partial T}{\partial t} + u \frac{\partial T}{\partial x} + v \frac{\partial T}{\partial y} = \alpha \frac{\partial^2 T}{\partial y^2} - \frac{1}{\rho C_p} \frac{\partial q_r}{\partial y} \tag{4}$$

The boundary conditions of the problem are defined as follows

$$u = U_w, v = 0, T = T_w, N = 0 \text{ at } y = 0 \tag{6}$$

$$u \rightarrow 0, N \rightarrow 0, T \rightarrow T_\infty, \frac{\partial u}{\partial y} \rightarrow 0 \text{ at } y \rightarrow \infty \tag{7}$$

where u and v are the velocity components in the x and y directions, respectively, λ_1 is the ratio of the relaxation and retardation times, λ_2 is the relaxation time, T is the fluid temperature, $\nu = \frac{\mu}{\rho}$ is the kinematic viscosity, μ is the coefficient of fluid viscosity, ρ is the fluid density, K is the permeability of the porous medium, κ is thermal conductivity of fluid, q_r is the radiative heat flux, C_p is specific heat at constant pressure, and α is thermal diffusivity. Using Roseland approximation, we have $q_r = -\frac{4\sigma_0}{3k_0} \frac{\partial T^4}{\partial y}$ where σ_0 is the Stefan Boltzmann constant, and k_0 is the mean absorption coefficient. Assuming that the difference in temperature within the

The sheet velocity and temperature are U_w and T_w , respectively, and are assumed to be

$$U_w = \frac{ax}{(1-ct)}, T_w = T_\infty + \frac{bx}{(1-ct)^2}, \text{ } b \text{ and } c \text{ are positive constants} \tag{8}$$

Hereby introducing the following Similarity variables, we have

$$\eta = y \sqrt{\frac{a}{\nu(1-ct)}}, \psi = \sqrt{\frac{\nu a}{(1-ct)}} x f(\eta), N = \sqrt{\frac{\alpha^3}{\nu(1-ct)^3}} x g(\eta), \theta(\eta) = \frac{T - T_\infty}{T_w - T_\infty} \tag{9}$$

$$u = \frac{\partial \psi}{\partial y}, v = \frac{\partial \psi}{\partial x}$$

where $\psi = \psi(x, y, t)$ is the stream function.

Using Similarity Transformations (9), Eqs. (2), (3) and (4) become

$$\begin{aligned} (1 + A_1) f'''' + (1 + \lambda_2) \left[f f''' - (f')^2 \right] - A \left(f' + \frac{1}{2} \eta f'' \right) + \xi \theta - M f' + A_1 g' - K_p f' \\ + (1 + A_1) \beta \left[(f')^2 - f f'''' + A \left(2f''' + \frac{1}{2} \eta f'''' \right) \right] = 0 \end{aligned} \tag{10}$$

$$\lambda_0 g'' + fg' - f'g - \frac{A}{2}(3g + \eta g') - A_1 B(2g + f'') = 0 \quad (11) \quad \frac{1}{2}(1 + \lambda_2)C_f \sqrt{R_e} = -[f''(0) + \beta f'(0)f''(0) - f(0)f'''(0) + A_1 g(0)] \quad (15)$$

$$\left(\frac{1}{Pr} + R\right)\theta'' + f\theta' - f'\theta - A\left(2\theta + \frac{1}{2}\eta\theta'\right) = 0 \quad (12) \quad \frac{Nu}{\sqrt{R_e}} = -\theta'(0) \quad (16)$$

where

$$A = \frac{\zeta}{a}, \xi = \frac{G_c}{R_c^2}, G_r = \frac{g\beta_f(T_w - T_\infty)x^3}{\nu^2}, R_e = \frac{U_b x}{\nu}, \beta = \frac{\lambda_1 a}{1 - ct},$$

$$M = \frac{\sigma B_0^2(1 - ct)}{\rho a},$$

$$A_1 = \frac{K}{\mu}, \lambda_0 = \frac{\gamma}{\mu j}, B = \frac{\nu(1 - ct)}{ja}, K_p = \frac{\nu(1 - ct)}{aK_*}, \text{ and } Pr = \frac{\nu}{\alpha}, R = \frac{16\sigma_0 T_\infty^3}{3\mu c_p K_1}$$

The non-dimensional parameters $A, \xi, \beta, M, \lambda_0, B$ and A_1 represent unsteadiness parameter, mixed convection parameter, Deborah number, magnetic field parameter, spin gradient viscosity parameter, micro-coupling parameter, respectively, whereas K_p, Pr and R represent porosity parameter, Prandtl number and Radiation Parameter, respectively.

The Deborah number [30] characterizes fluidity of materials under specific flow conditions. The fluidity of materials (solid-like and fluid-like) depends on relaxation time and observation time. The larger Deborah number, the more solid the material and the smaller the Deborah number, the more fluid the material.

Boundary conditions defined by (6) and (7) are uniquely formed in similarity as follows

$$f(0) = 0, f'(0) = 1, \theta(0) = 1, g(0) = 0, \text{ at } \eta = 0 \quad (13)$$

$$f'(\eta) \rightarrow 0, g(\eta) \rightarrow 0, \theta(\eta) \rightarrow 0, f''(\infty) \rightarrow 0 \text{ at } \eta \rightarrow \infty \quad (14)$$

Some of essential physical parameters, like skin coefficient and Nusselt's number, which are defined as.

$$C_f = \frac{2T_w}{\rho U_b^2} \text{ and } Nu = \frac{q_w}{T_w - T_\infty} \left(\frac{x}{k}\right). \text{ Where } T_w = -\frac{\mu}{1 + \lambda_2}$$

$$\left[\frac{du}{dy} + \lambda_1\left(u\frac{\partial^2 u}{\partial x \partial y} + v\frac{\partial^2 u}{\partial y^2}\right) + \frac{K}{\mu}(N)\right] \text{ at } y=0 \text{ and } q_w = -\left(\frac{dT}{dy}\right)$$

at $y=0$.

Therefore,

$$y_4' = \frac{1}{\left(y_1 - \frac{1}{2}A\eta\right)} \left[y_3^2 + 2Ay_4\right] + \frac{1}{(1 + A_1)\beta\left(y_1 - \frac{1}{2}A\eta\right)} \left\{(1 + A_1)y_4 + (1 + \lambda_2)\left\{y_1y_3 - y_2^2 - A\left(y_2 + \frac{1}{2}\eta y_3\right) + \xi y_7 - My_2 + A_1y_6 - K_p y_2\right\}\right\} \quad (21)$$

The Nusselt number N_u appearing in Eq. (16) gives the basic difference between convection and conduction heat transfer across a boundary. The larger the Nusselt

number, the more effective the convection. If $N_u = 1$, then the heat transfer across the boundary is through by pure conduction.

Solution algorithm

To solve boundary value problems (10)-(12) with boundary conditions given by (13) and (14), we used built-in solver called `bvp4c` function in MATLAB software package. The algorithm is based on to reduce the nonlinear ordinary differential Eqs. (10)-(12) with boundary conditions (13) and (14) into the system of first order nonlinear differential equations as follows

$$f = y_1, f' = y_2, f'' = y_3, f''' = y_4, g = y_5, g' = y_6, \theta = y_7, \theta' = y_8 \quad (17)$$

Using (15), Eqs. (8)-(10) reduce to first order nonlinear ordinary differential equations

$$y_1' = y_2 \quad (18)$$

$$y_2' = y_3 \quad (19)$$

$$y_3' = y_4 \quad (20)$$

Fig. 2 Simulated velocity for various values of porosity parameter

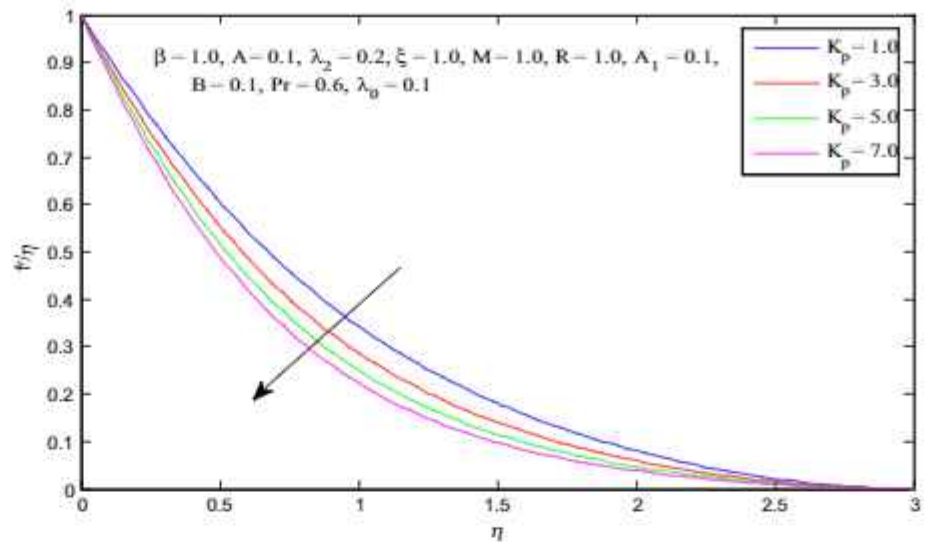
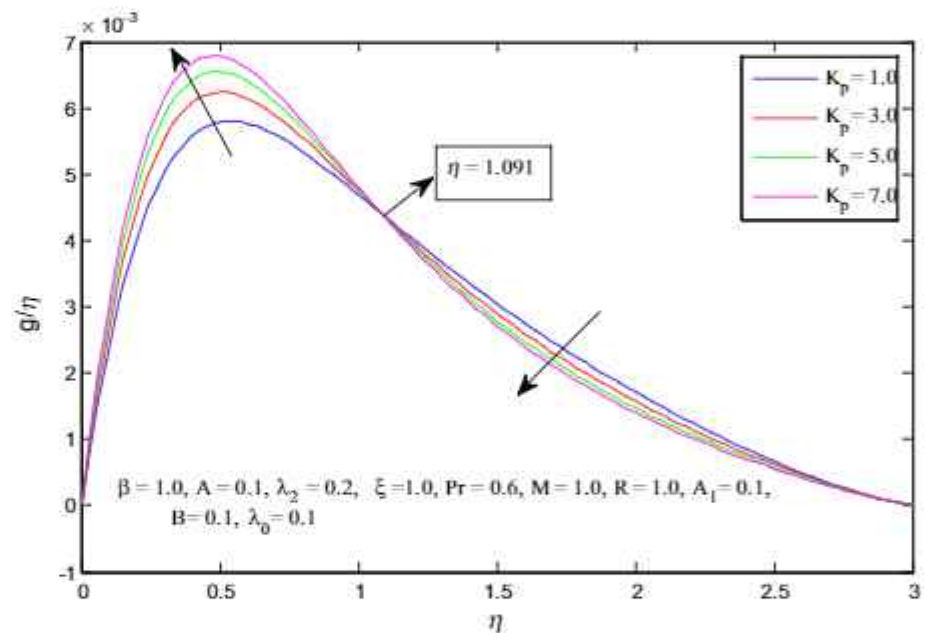


Fig. 3 Micro-rotation for different values of porosity parameter



$$y'_5 = y_6 \tag{22}$$

$$y'_6 = \frac{1}{\lambda_0} \left[y_2 y_5 - y_1 y_6 + \frac{A}{2} (3y_5 + \eta y_6) + A_1 B (2y_5 + y_3) \right] \tag{23}$$

$$y'_7 = y_8 \tag{24}$$

$$y'_8 = \frac{1}{\left(\frac{1}{Pr} + R \right)} \left[y_2 y_7 - y_1 y_8 + A \left(2y_7 + \frac{1}{2} \eta y_8 \right) \right] \tag{25}$$

The boundary conditions yield

$$\begin{aligned} y_1(0) = 0, y_2(0) = 1, y_3(0) = 0, y_7(0) = 1, \eta = 0 \\ y_2(\eta) \rightarrow 0, y_3(\eta) \rightarrow 0, y_5(\eta) \rightarrow 0, y_7(\eta) \rightarrow 0 \text{ at } \eta \rightarrow \infty \end{aligned} \tag{26}$$

Above system of ordinary nonlinear ordinary differential Eqs. (18)-(25) with boundary Eq. (26) are solved by built-in function bvp4c in MATLAB software.

Graphical results and discussion

The objective of the present paper is to study the unsteady heat transfer in MHD micropolar Jeffery Fluid flow through porous medium over a stretching sheet. The numerical

Fig. 4 Temperature profile for different values of porosity parameter

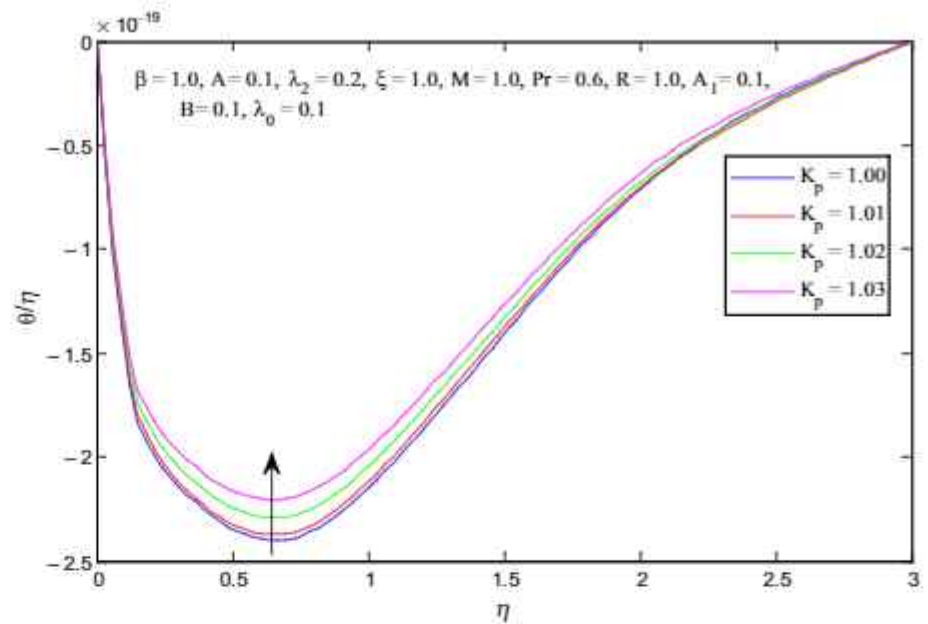
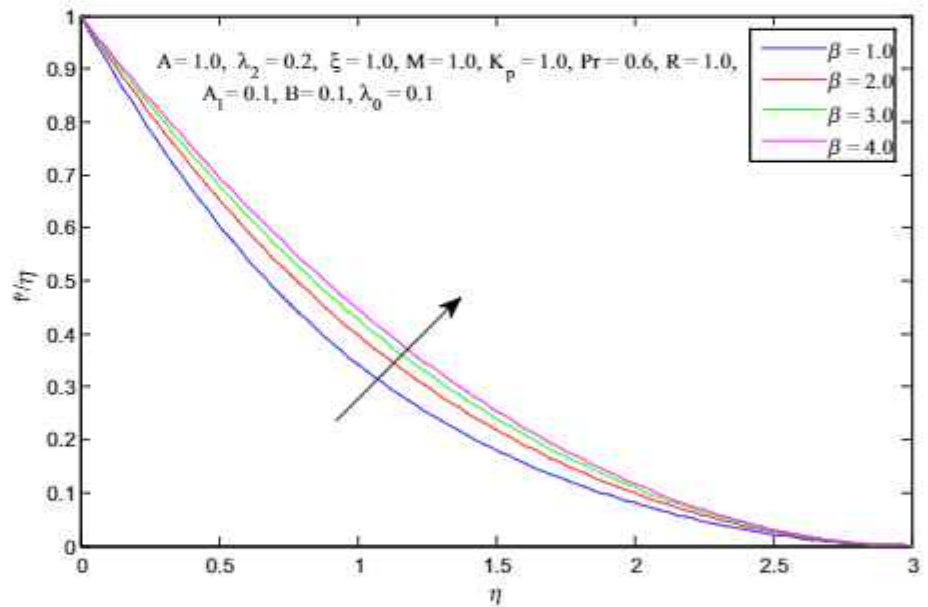


Fig. 5 Simulated velocity profile for various values of Deborah number

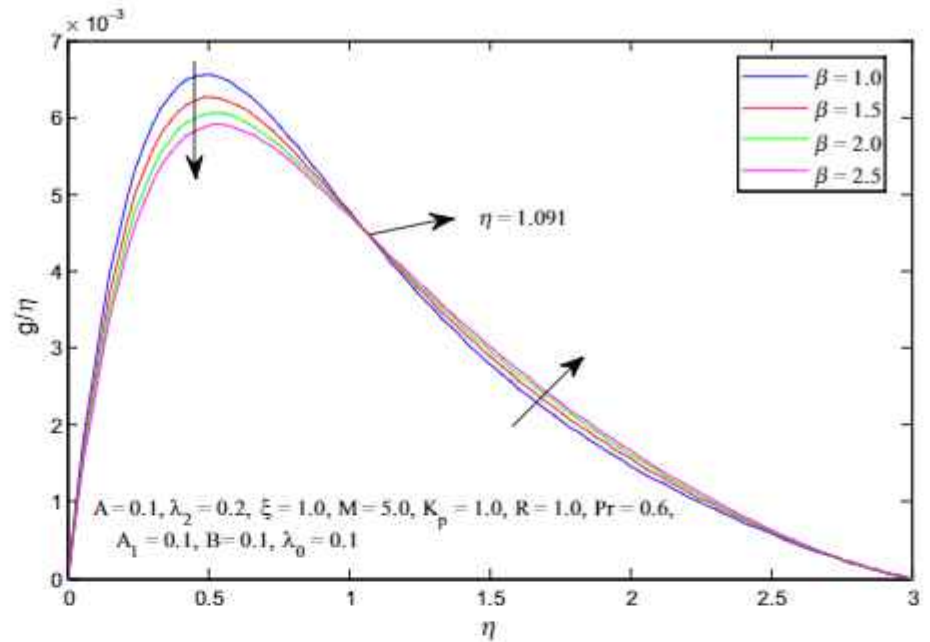
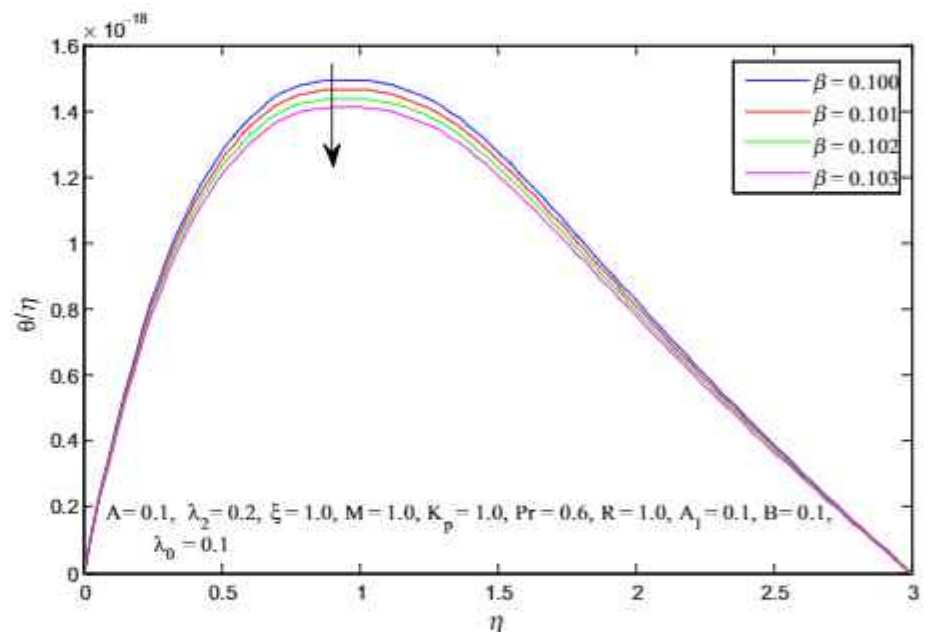


solutions, obtained by *bvp4c* (boundary value problem fourth order Runge–Kutta collocation method) built-in function in MATLAB software, are performed for different values of dimensionless parameter involved in the equations such as the Jeffrey parameter, Magnetic parameter, mixed convection parameter, radiation parameter, etc. The impact of pertinent parameters on the velocity, temperature and micro-rotation is been discussed in this section. To illustrate the computed results, some figures are plotted and explained.

Figures 2–4 depict the effect of porosity parameter on simulated velocity, micro-rotation and temperature profiles. Simulated velocity decreases while temperature increases

with increase in value of porosity parameter. That is, porous medium resists the flow while the larger the denseness of porous medium the larger the temperature. The micropolar particles of the fluid oscillates irregularly about the point $\eta = 1.091$.

Figures 5–7 exhibit the influence of Deborah number on the distribution of velocity, micro-rotation and temperature profiles, respectively. Simulated velocity increases while temperature decreases with increase in Deborah number. The higher value of Deborah number indicates that flow is strong enough which led to the fluid to become stretched and highly oriented in one direction. Angular velocity decreases

Fig. 6 Micro-rotation for various values of Deborah number

Fig. 7 Temperature profile for various values of Deborah number


first (near the surface) with the increase in Deborah number then after when $\eta = 1.091$ i.e., for bigger η (boundary layer thickness) trend is opposite. It happened as for smaller values of Deborah number; the behavior of fluid tends to be viscous.

Figures 8–10 display the effect of different values of unsteadiness parameter on simulated velocity, micro-rotation and temperature profiles. Both simulated velocity and temperature increases with the increasing values of unsteadiness parameter while a decrease is observed in

micro-rotation profile in the beginning. After η attains a value of 0.8182 angular velocity increases.

Figures 11–13 show that simulated velocity decreases while temperature increases with increase in value of Jeffery fluid parameter and micro-rotation profile first increases and then decreases after $\eta = 1.152$.

Figures 14, 15 exhibit the effect of mixed convection parameter on simulated velocity and temperature profile. With increase in the value of mixed convection leads to the decrease in temperature and simulated velocity profile.

Fig. 8 Simulated velocity for various values of unsteadiness parameter

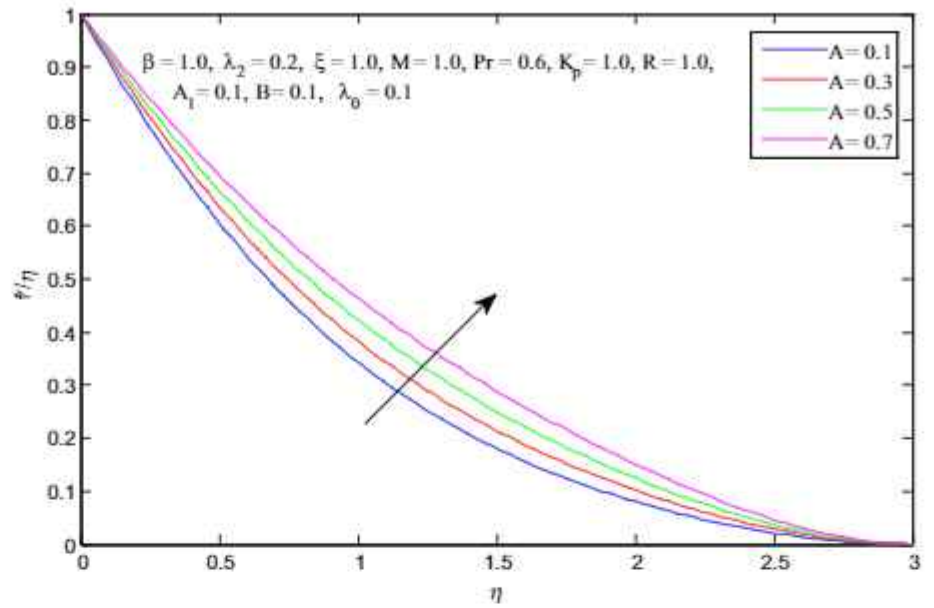


Fig. 9 Micro-rotation for different values of unsteadiness parameter

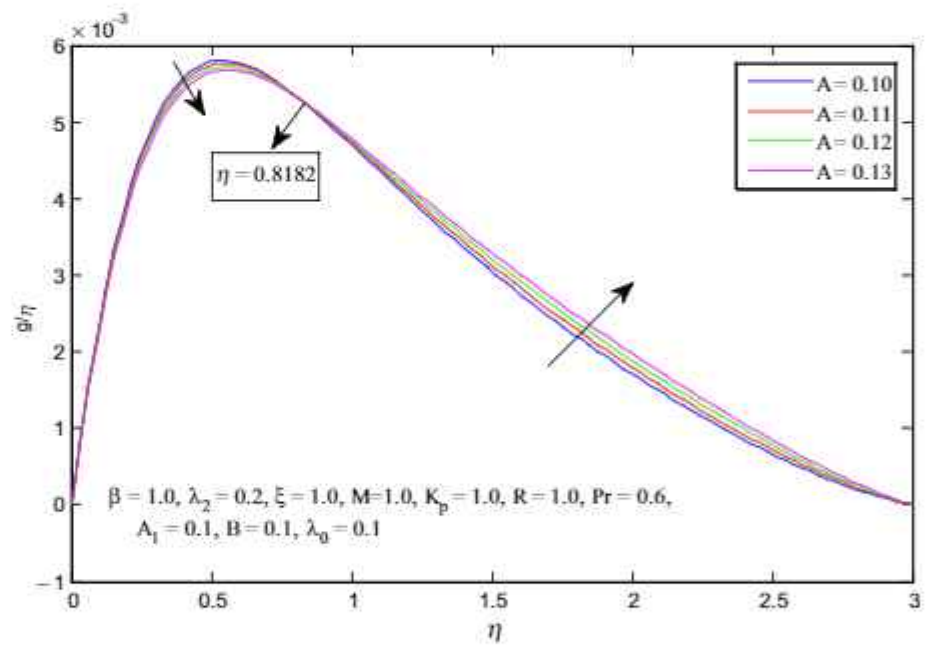


Fig. 10 Temperature profile for various values of unsteadiness parameter

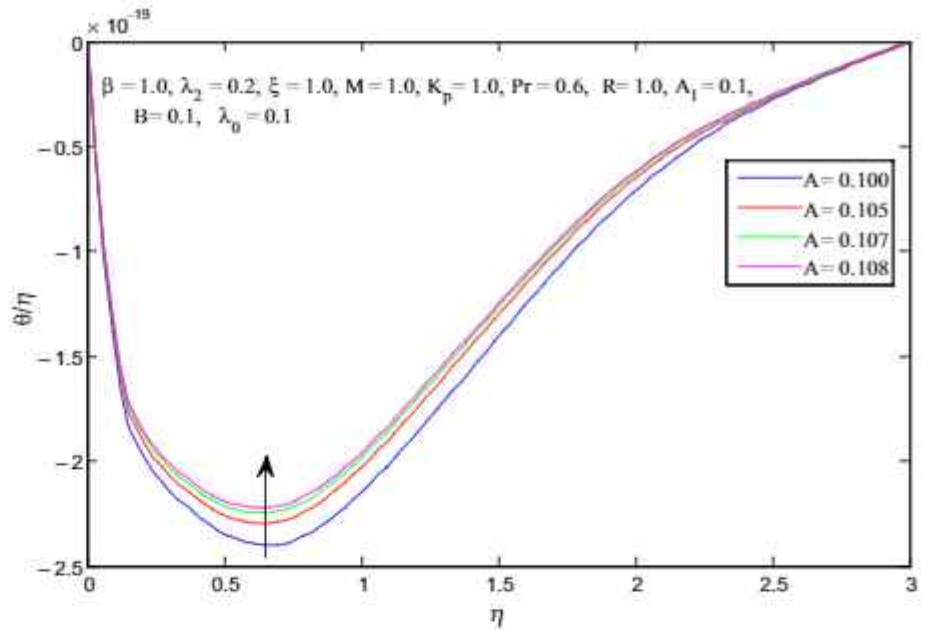


Fig. 11 Simulated velocity for various values of Jeffrey fluid parameter

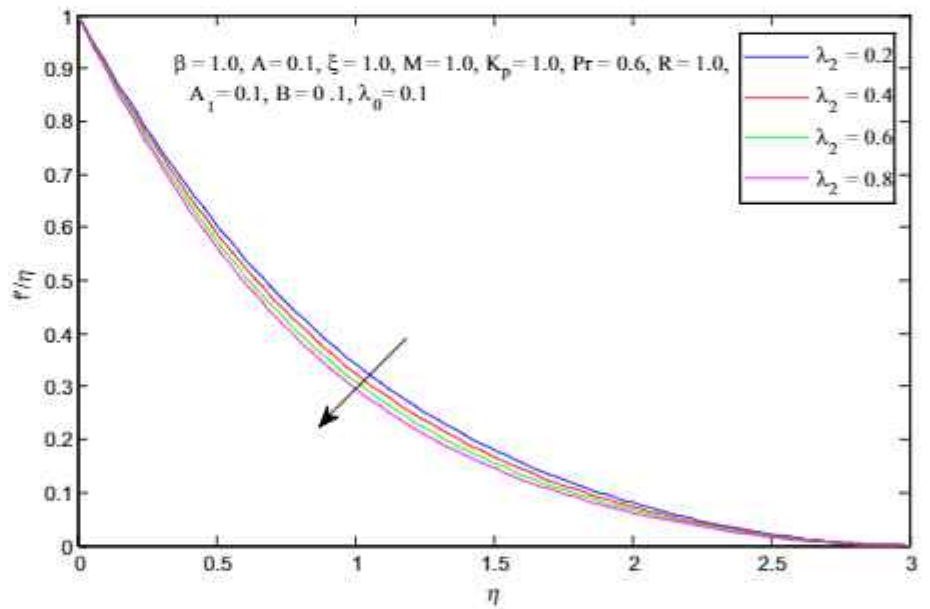


Fig. 12 Micro-rotation for different values of Jeffrey fluid parameter

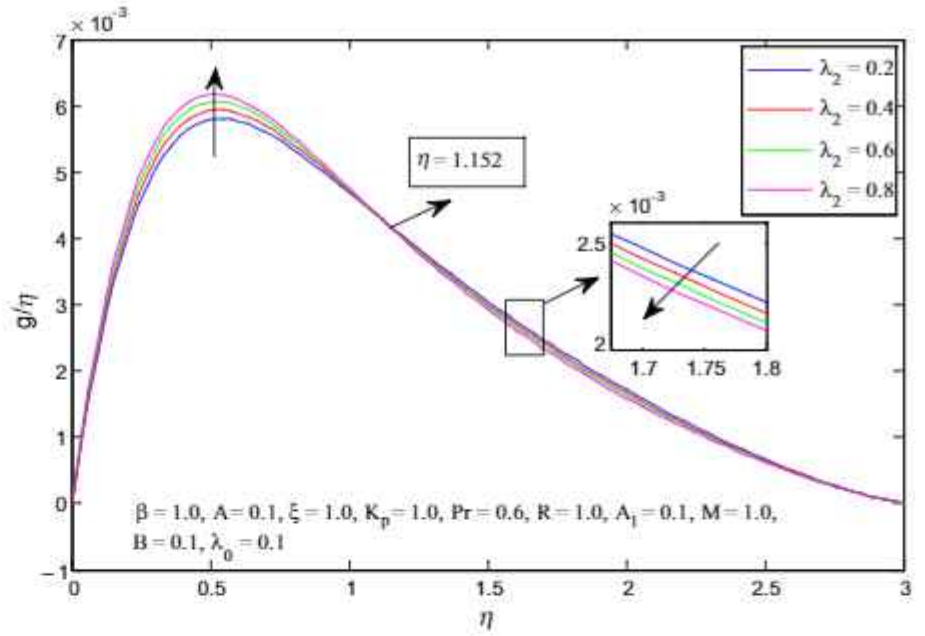


Fig. 13 Temperature profile for various values of Jeffrey fluid parameter

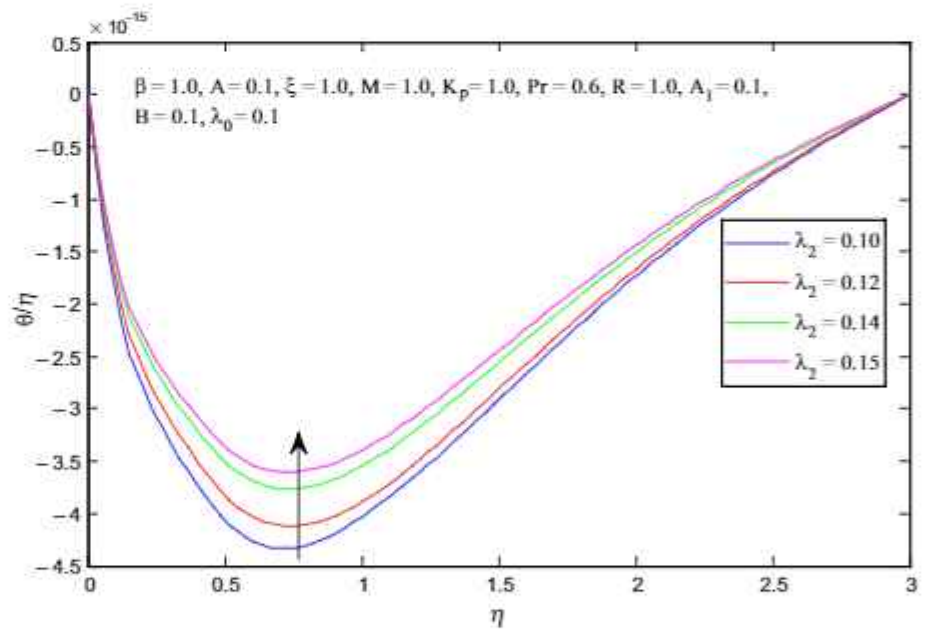


Fig. 14 Simulated velocity for various values of mixed convection parameter

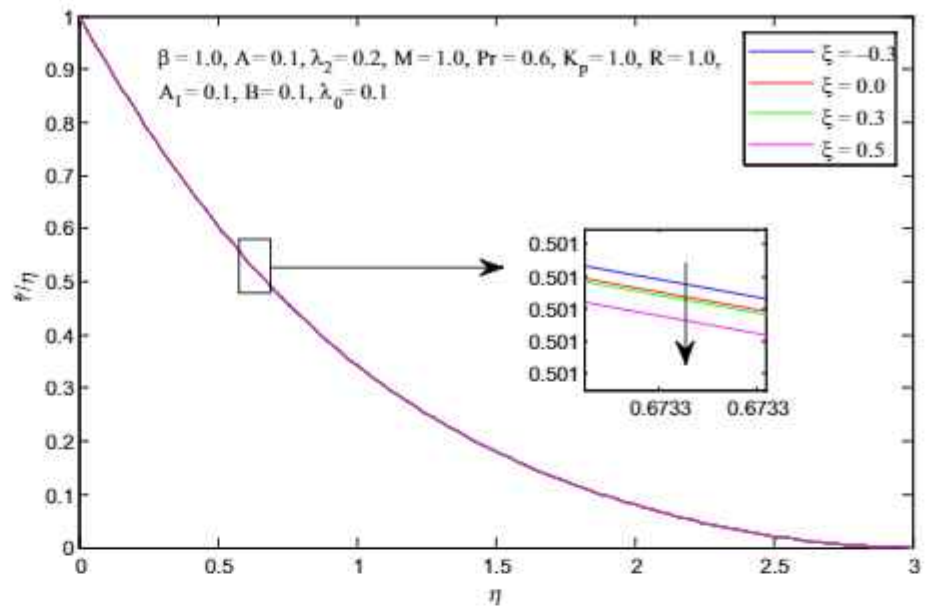


Fig. 15 Temperature profile for various values of mixed convection parameter

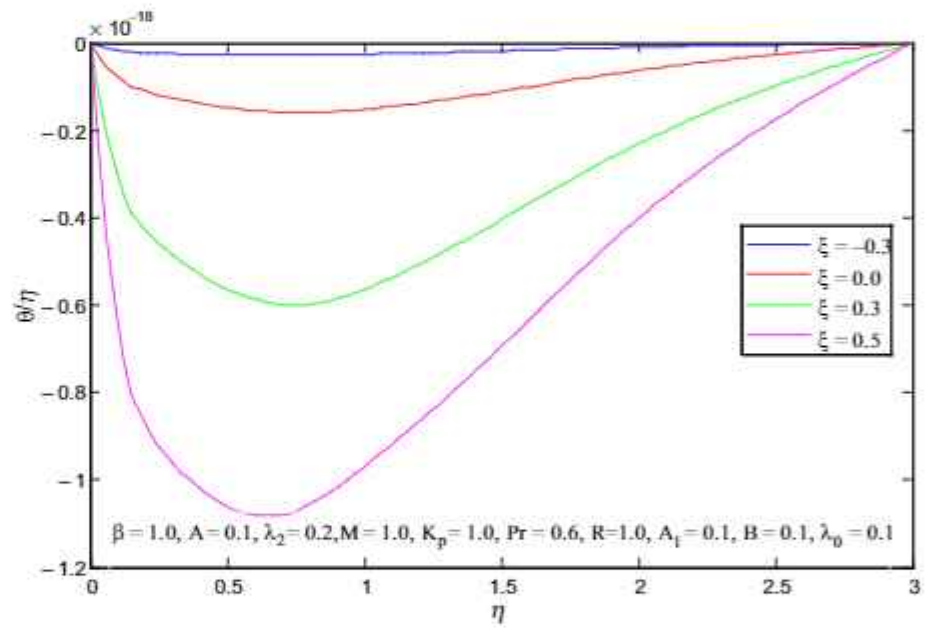


Fig. 16 Simulated velocity for various values of magnetic field parameter

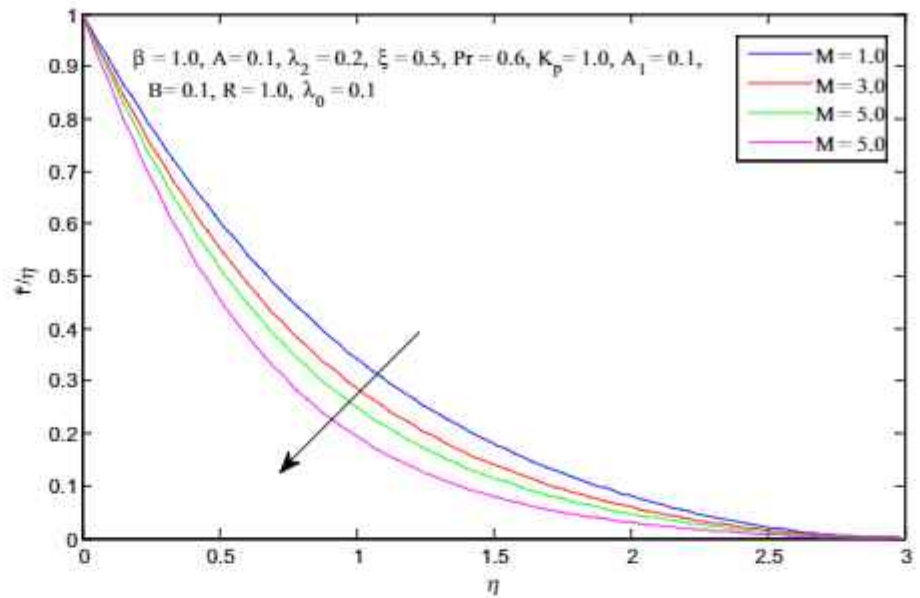
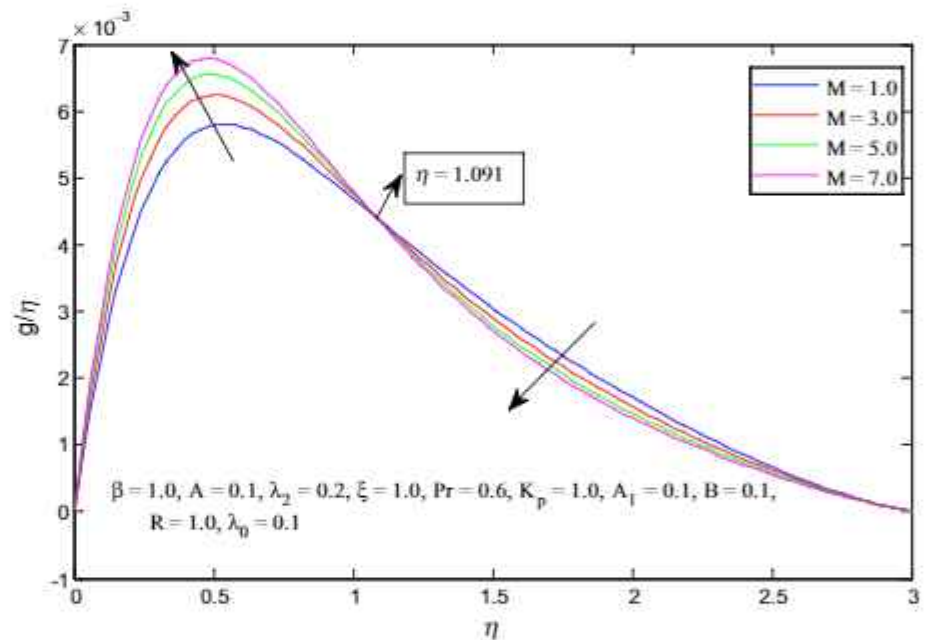


Fig. 17 Micro-rotation for different values of magnetic field parameter



From Figs. 16–18, it is seen that the simulated velocity decreases while temperature (near surface) of fluid increases with increase in value of magnetic field parameter. The presence of MHD deferred the development of liquid, however offered more energy stockpiling in the actual liquid. Angular velocity increases first with the increase in magnetic field parameter then after when $\eta = 1.091$ a reverse effect is observed.

Figures 19–21 demonstrated that both simulated velocity and temperature decreases with the increasing values of coupling parameter while angular velocity increases.

Figures 22–24 reveal that increase in micro-inertia density parameter leads to decrease in temperature and simulated velocity profile and increase in micro-rotation profile.

Figures 25–27 remarked that as the values of spin gradient viscosity parameter increases the temperature profile decreases and simulated velocity increases. Angular velocity decreases first with the increase in spin gradient viscosity then after when $\eta = 1.091$ an increase in shown in micro-rotation profile.

Figures 28, 29 clearly depicted that temperature distribution decreases with the increase in radiation parameter while micro-rotation profile shown an increase.

Fig. 18 Temperature profile for various values of magnetic field parameter

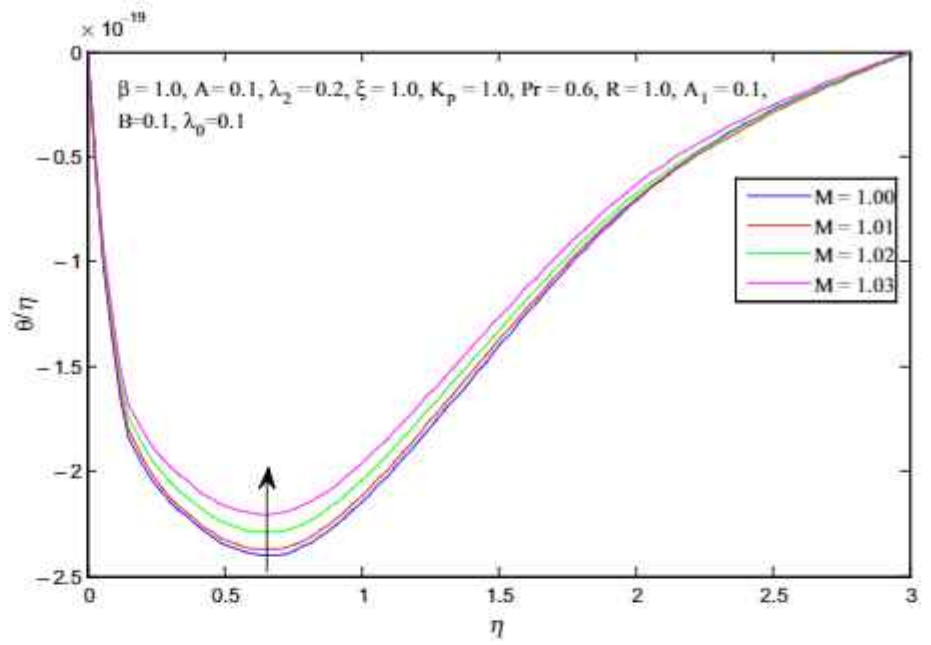


Fig. 19 Simulated velocity for various values of coupling parameter

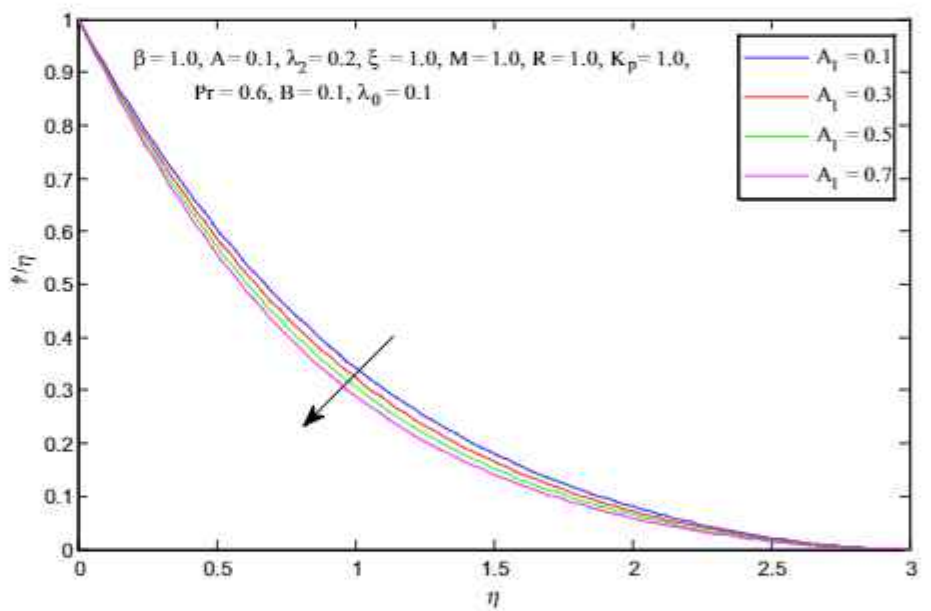


Fig. 20 Micro-rotation for different values of coupling parameter

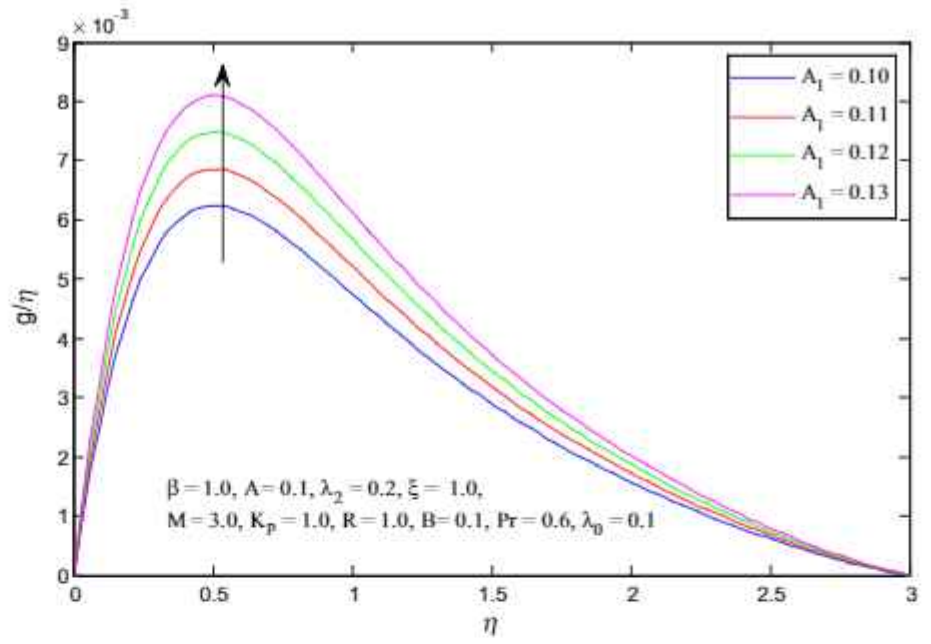


Fig. 21 Temperature profile for different values of coupling parameter

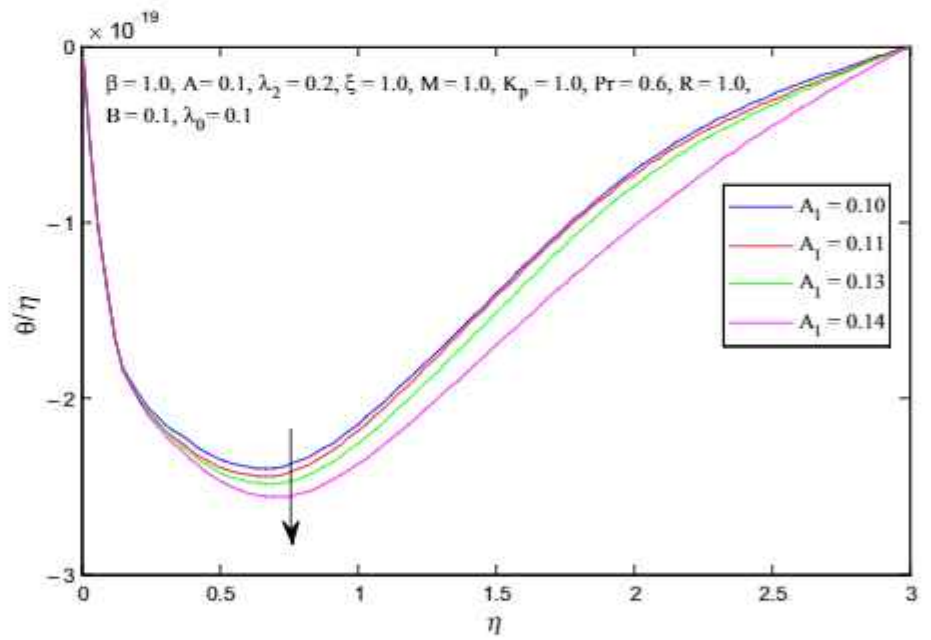


Fig. 22 Simulated velocity for various values of micro-inertia density parameter

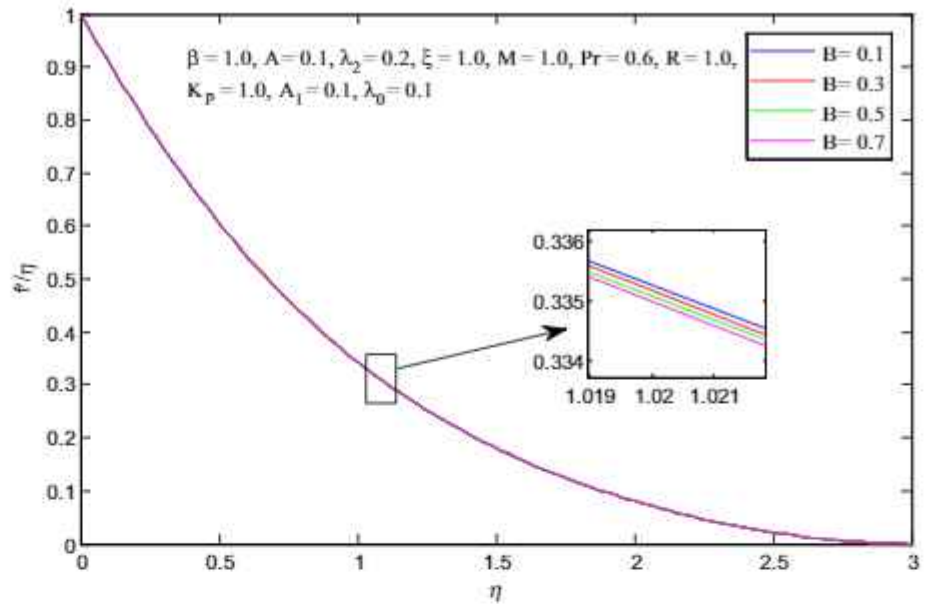


Fig. 23 Micro-rotation for various values of micro-inertia density parameter

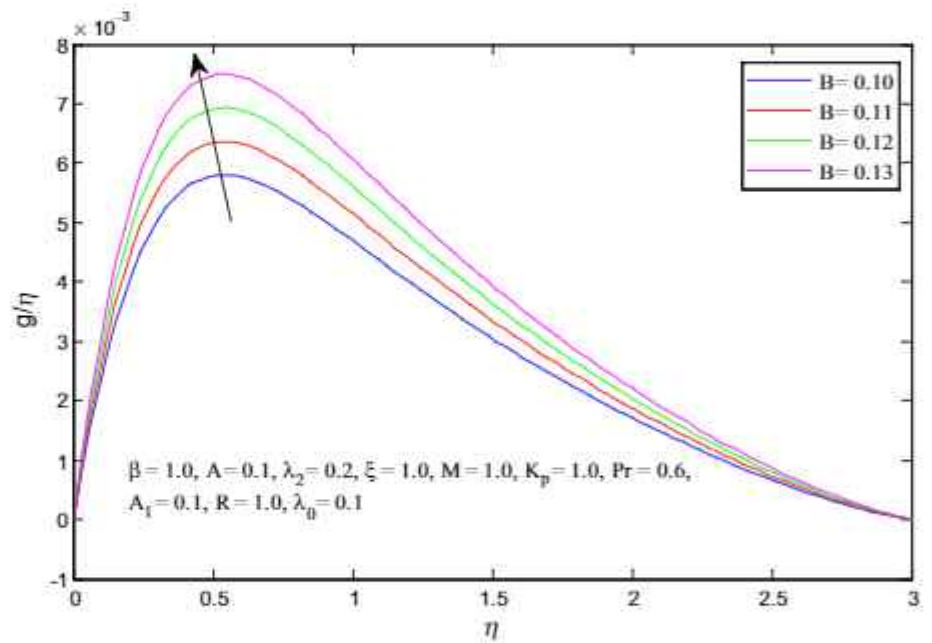


Fig. 24 Temperature profile for different values of micro-inertia density parameter

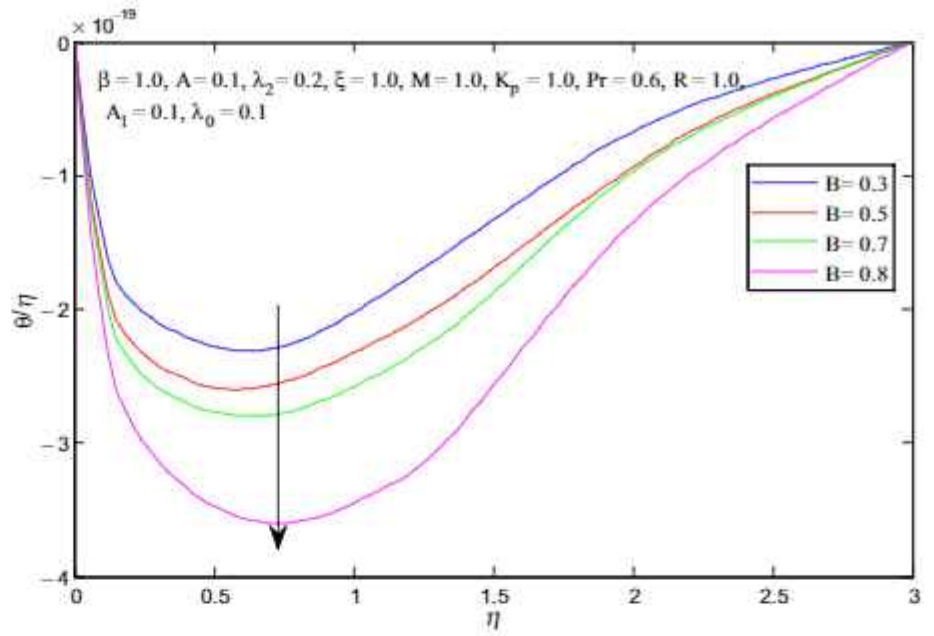


Fig. 25 Simulated velocity for different values of spin gradient viscosity parameter

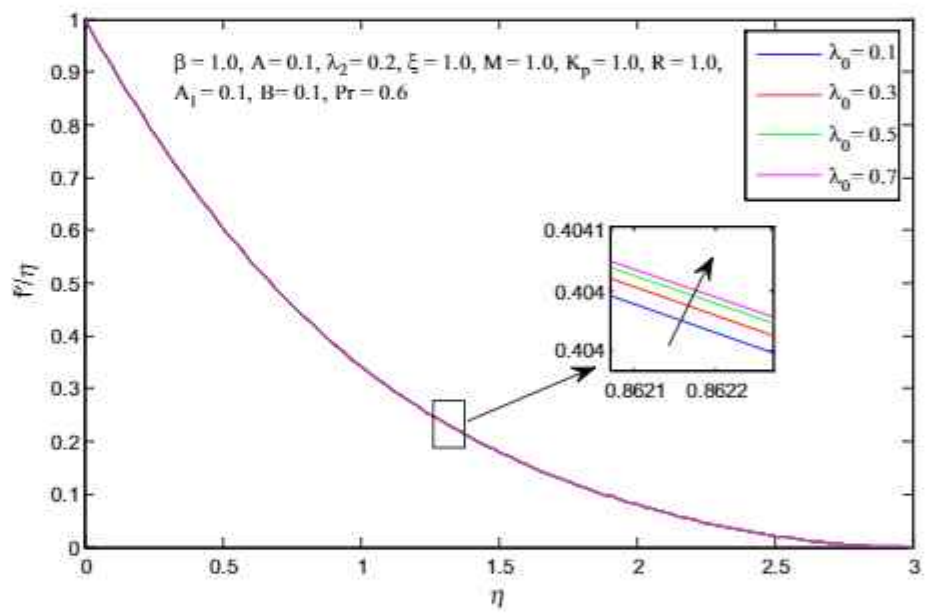


Fig. 26 Micro-rotation for various values of spin gradient viscosity parameter

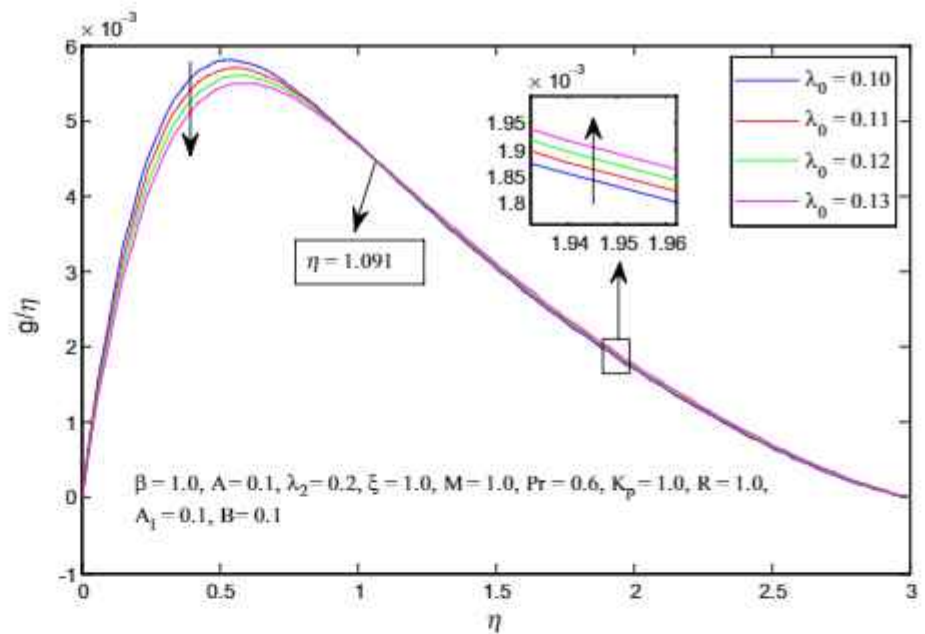


Fig. 27 Temperature profile for various values of spin gradient viscosity parameter

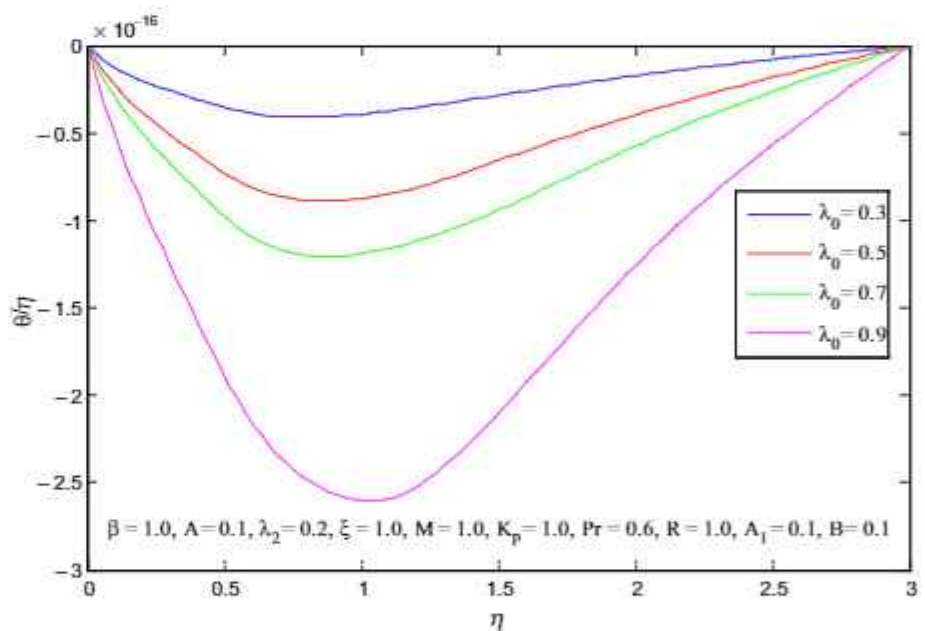


Figure 30 It is been observed that for increasing values of Prandtl number there is a decrease in temperature profile. It happened as higher of Prandtl number have the weaker diffusivity. The viscosity is dominant, and thus, heat transfer is less convective.

It has been observed from Figs. 31, 32 that the skin friction coefficient against Deborah number decreases

with increasing the values of Jeffrey fluid parameter which shows that near stretching sheet friction is reduced in the presence of Jeffrey fluid whereas it increases against Jeffrey fluid parameter as the Deborah number increases, and from Figs. 33, 34, it is shown that the Nusselt number decreases with increasing the values of Jeffrey fluid parameter while it increases in increasing the values of Deborah number.

Fig. 28 Micro-rotation for various values of radiation parameter

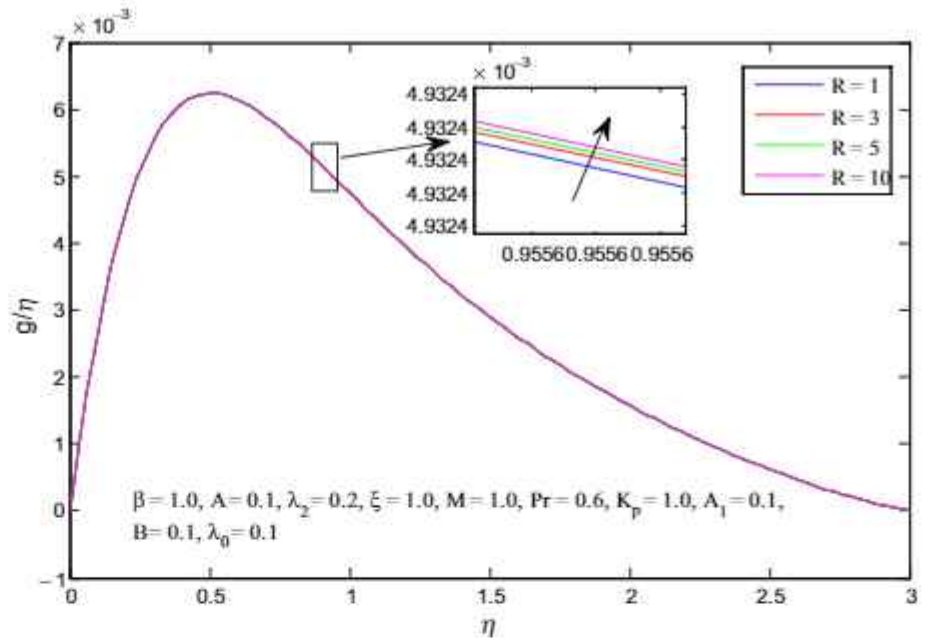


Fig. 29 Temperature profile for different values of radiation parameter

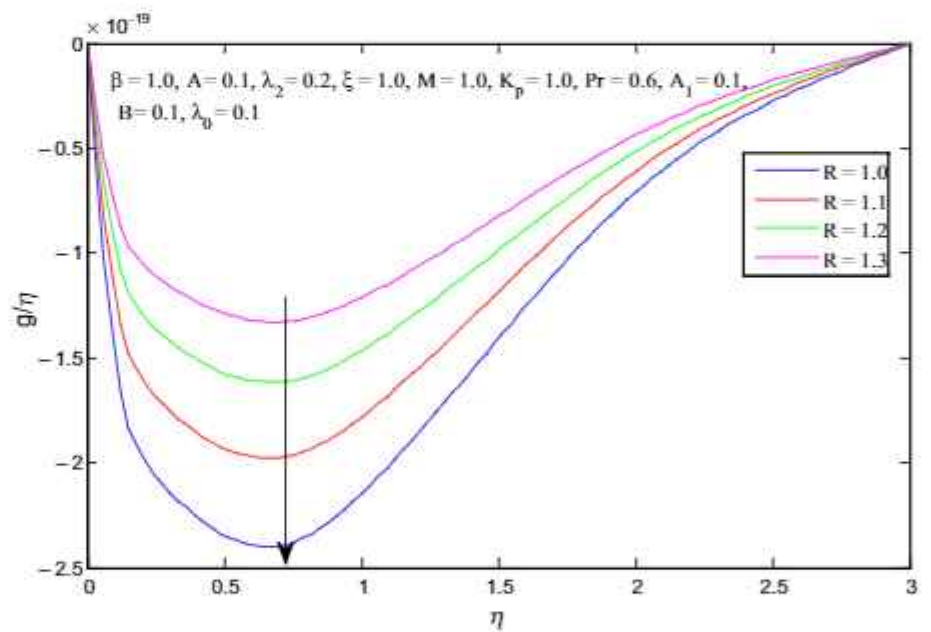


Fig. 30 Temperature profile for different values of Prandtl number

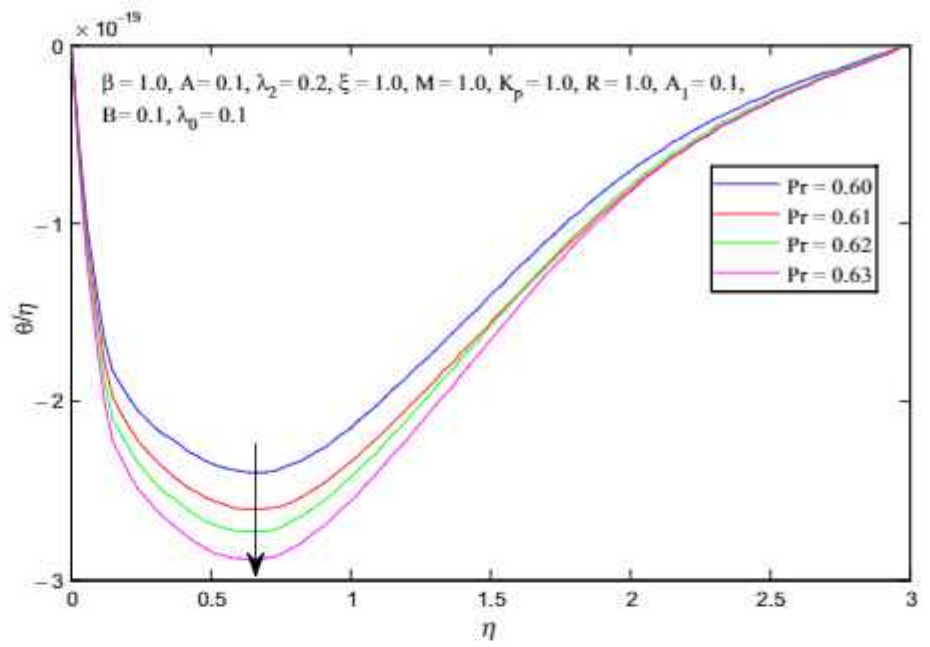


Fig. 31 Variation of skin friction coefficient against Deborah number

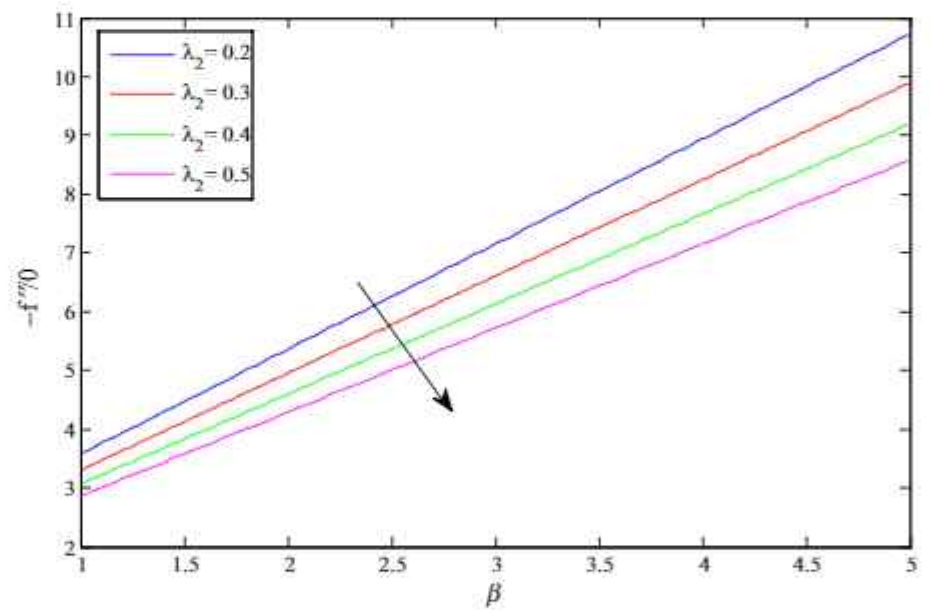


Fig. 32 Variation of skin friction coefficient against Jeffrey fluid parameter

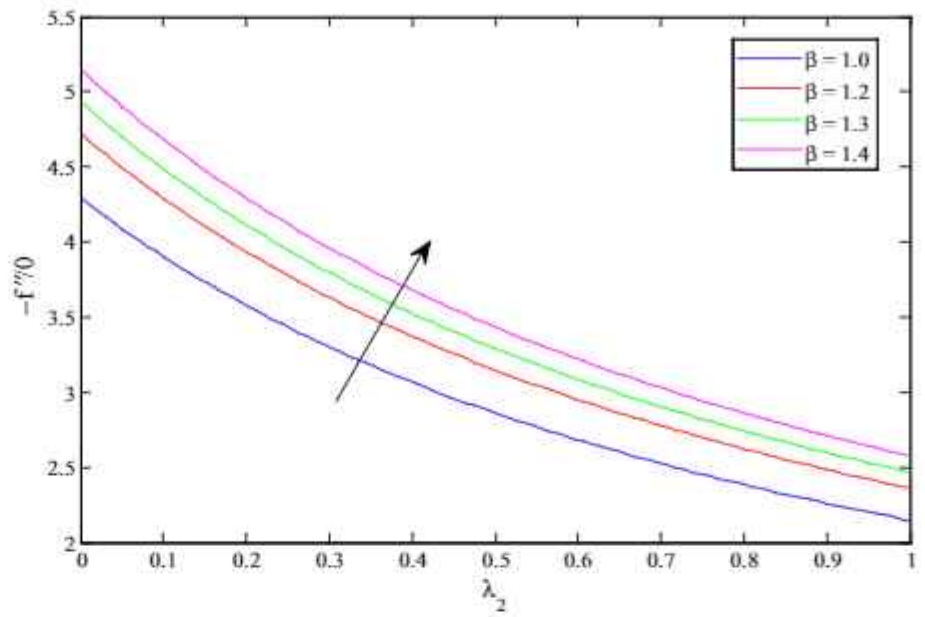


Fig. 33 Variation of Nusselt number against Deborah number

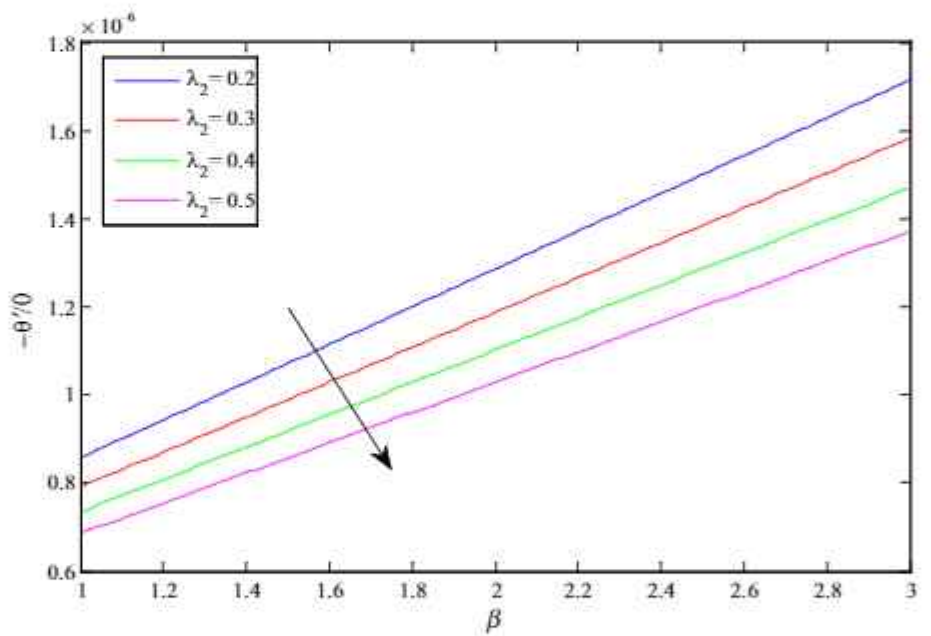
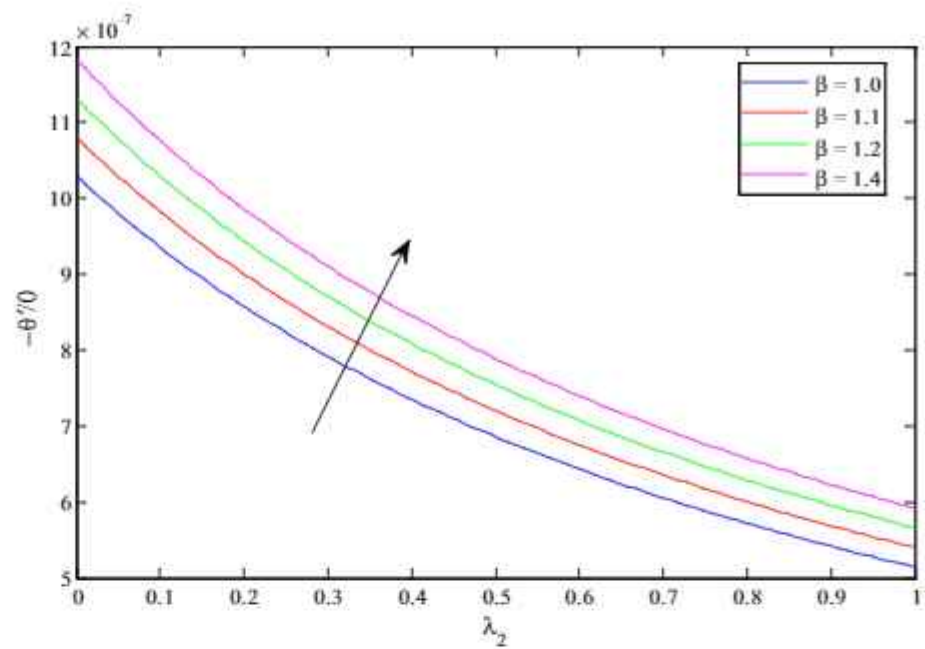


Fig. 34 Variation of Nusselt number against Jeffrey fluid parameter



Conclusions

The following points are quite evident from the graphical results as discussed above in the numerical investigation of MHD Micropolar Jeffrey Fluid flow through porous medium over a stretching sheet:

- Simulated velocity of the fluid decreases with the increasing the values of Jeffrey fluid parameter, porosity parameter, mixed convection parameter, coupling parameter, micro-inertia density parameter and magnetic field parameter while trend is opposite in case of Deborah number, spin gradient viscosity parameter and unsteadiness parameter.
- The parameters like Jeffrey fluid, Magnetic field, porosity, coupling, Deborah number and spin gradient viscosity parameter have reverse effect on temperature profile.
- The larger the Prandtl number smaller the temperature of the fluid.
- Near the surface, the larger values of Deborah number and spin gradient viscosity parameter led to decrease the distribution of micro-rotation of fluid but after $\eta > 1.091$ it enhances. That is, the larger the Deborah number, the micro-particles of the fluid behave as solid-like resulting decreases the velocity of the micro-particles when $\eta < 1.091$. But an exactly reverse effect is shown on micro-rotation against the porosity parameter and magnetic field.
- A dual effect is also shown by Jeffrey fluid parameter on micro-rotation profile. Near the surface, it increases and then decreases after $\eta > 1.152$.

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