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Transmission, survival and disease cycle of *Colletotrichum capsici f. sp. cyamopsicola* cuasing anthracnose of guar

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ABSTRACT

In the present work, anthracnose (*colletotrichum capsici f.sp. cyamopsicola* of guar was studied in the Plant Pathology Laboratory, Department of Pathology, College of Agriculture, Gwalior. Survival of the*Colletotrichum capsici* f. sp. *cyamopsicola* was examined by inserting the parts of leaves packed in paper envelop and buried at varied depth *viz.,*5,10,15, 20 and 25 cm in pots filled with sterilized soil. Even after 12 months, the fungus was found viable at 5 and 10 cm depth. Survivability of the pathogen in plant debris at 15, 20, 25 cm depth was lost within 8 months. It was found that the fungus was internally seed borne. The pathogen was located in the seed coat. The fungus was found to be transmitted from seed to plant and from plant to seed.

Figure : 01 References : 22 Tables : 03
KEY WORDS : *Colletotrichum capsici,* Guar, Survival

Introduction

Cluster bean (*Cyamopsis tetragonoloba*) is an important multi-use leguminous crop, grown in arid and semi-arid regions during the kharif season. Guar is a deep rooted,drought and high temperature tolerant plant[10]. Eighty percent of the world's guar production is produced in India[18].Its annual production is about 2.7 million metric tons, in india. Rajasthan is the largest producer of guar in India. Other guar producing states are Haryana, Gujarat, UP, Punjab, Madhya Pradesh *etc.* Guar is mostly produced in Gwalior, Shivpuri, Morena, and Bhind in Madhya Pradesh.The cluster bean is an important source of gum which is extracted from guar seeds. Gum is used in a variety of foods such as ice cream, baked goods, and dairy products. moreover, Its gum is utilised in a variety of industries, including pharmaceuticals, cosmetics, mining, textiles, paper and oil drilling. The cluster been's pods are utilised for vegetable purposes. Its plant, seed and straw are the nutritious fodder and used to feed the livestock . Guar crop is susceptible to a number of disease like Powdery mildew (*Leveillula taurica*), Alternaria leaf blight *(Alternaria cyamopsidis*) and Anthracnose caused by *Colletotrichum capsici f.sp. cyamopsicola*[3]. Anthracnose is a sever disease in guar growring areas. Every year, the crop suffers significant losses owing to the anthracnose disease.During the Kharif season, this disease causes black patches on the leaves, petioles, and stem.

Material and Methods

Transmission of the fungus on the host from one season to another season was studied.

(a) Seed to plant

(i) Survival of *Colletotrichum capsici* f. sp. *cyamopsicola* on infected plant debris

Infected crop Debris (Twigs,Stem and Leaves) were collected from the field and cut into small pieces which were placed in pots and buried at different depths 5 ,10, 15 ,20,25 cm. Pots filled with field soil. Isolation of the fungus from buried crop debris was done at monthly intervals. Observations were recorded on the survival and viability of the fungus.

(ii) Location of fungus inside the guar seed

Component plating was used to investigate the

TABLE -1: Survival of *Colletotrichum capsici* f. sp. *cyamopsicola* in/on infected crop debris buried in soil (Post crop season)

Time interval (days)	Month	Different depths of placement (cm)				
		5	10	15	20	25
30	November	+	+	+	+	+
60	December	+	+	+	+	+
90	January	+	+	+	+	+
120	February	+	+	+	+	+
150	March	+	+	+	+	+
180	April	+	+	+	+	+
210	May	+	+	+	+	+
240	June	+	+	+	+	+
270	July	+	+	-	-	-
300	August	+	+	-	-	-
330	September	+	+	-	-	-
360	October	+	+	-	-	-

+ Survival

- No Survival

site of infection of *C. capsici* f. sp. *cyamopsicola* on guar.

Component plating method

A total of twenty-five guar seeds were chosen at random. These seeds were soaked one in each glass tube containing sterilized water.Seeds were not allowed to come into contact with one another.Individual seed sections (seed coat, cotyledons, plumule and radicle) were plated on potato sucrose agar with the use of a forcep.These plates were cultured for 7 days at 25°C, after which the seed portions were tested for the presence of *C.capsici* f.sp*. cyamopsicola.*

(iii) Sand method

By spreading the seeds in sterilised sand filled in pots, the effect of seed borne *C. capsici* f.sp. *cyamopsicola* was also determined.Observations were recorded on the infection of seed and seedlings.

(b) Plant to seed

(i) Pod inoculation technique

Under field settings, guar pods of various ages of local cultivars were inoculated with spore suspension of a 10 days old culture of *C. capsici* f.sp. *cyamopsicola* and covered with polythene bags containing wet cotton to provide moisture and humidity for 6 days.

As a control, sterile water was sprayed on the pods. After surface sterilization, seeds from mature pods were collected and plated on PSA medium. Similarly, seeds from naturally infected pods were also collected and analyzed for fungal association using the blotter and

TABLE -2 : Association of *Colletotrichum capsici* f. sp. *cyamopsicola* with different seed components of Cluster bean

Seed components	Percent recovery from seed	
	Un-sterilized	Sterilized
Seed coat	20	18
Cotyledons	—	—
Redicle	—	—
Plumule	—	—

PSA methods. Infected plant parts such as stems, pods and leaves were gathered and examined under a microscope for fungus association before being isolated.

Result

The fungus was found transmitted from seed to plant and vice versa. The fungus was found associated with the seeds externally as well as internally with the leaves, stem and pods. Infected plant components that remain in the field act as infection sources.

Seed to plant

(a) Survival of *C. capsici* f.sp. *cyamopsicola* on infected plant debris

The fungus was found viable at 5 and 10 cm depths after 12 months (Table–1) At all depths of placement, an initial percent recovery was noted.However, the fungus' ability to survive in debris at15,20, and 25 cm deep was lost after 8 months, while it died after 12 months at 5 and 10 cm depth.

(b) Location of fungus inside the guar seed

The fungus could be seen as internally seed borne, according to the results of component plating of clusterbean seeds. In the seed coat, the pathogen was located (Table 2).

(c) Sand method

Guar seeds that were naturally affected were sown in pots containing sterile sand and the disease incidence was reported. Only 45% of the seeds germinated, 20% of the seeds decayed and 35 per cent post emergence mortality was observed (Table-3).

The reason of seed and seedling mortality was confirmed by plating the infected parts on PSA and *C. capsici* f.sp. *cyamopsicola* was isolated. Seed to seedling movement of the fungus was confirmed.

Plant to seed

In this procedure, guar pods were inoculated with spore suspension of *C. capsici* f. sp. *cyamopsicola* through spraying of spore suspension and covered with polythene bags containing wet cotton to provide humidity under field conditions. Seeds from mature pods were collected and plated on PSA medium both un-sterilized and surface sterilised.In comparison to sterilised seeds, it was observed that un-sterilized seeds had a greater infection rate.

Disease cycle of *Colletotrichum capsici* f. sp. *cyamopsicola*

Through infected seeds and crop leftovers, the causative fungus was able to persist from one crop season to the next. Once the diseased seeds started to sprout, the secondary inoculum originated from lesions that occurred on the cotyledons.

Almost all of the spores were carried by water. The hypocotyls and primary leaves served as secondary infection sites. Spattering rains associated with wind currents are the main source for the local dissemination of the pathogen. The pathogen passes the dry season in seeds and diseased plant parts. Survival upto 2 years in seeds has been observed. Damp, warm and high humidity conditions are necessary for anthracnose development. The causal agent survives as mycelium in the seed.

The spores after germination produced germ tube with appresoria penetrating directly into the epidermis later the mycelium develops both inter as well as intra-cellularly.In the beginning the hyphae grow rapidly inter-cellularly but cause little dis-coloration on the lower surface of the cotyledons. Leaf infection can occur at all stages of plant growth from seedling to adult plants

TABLE -3 : Role of seed borne *Colletotrichum capsici* f. sp. *cyamopsicola* in causing disease on guar seedlings in sand method

Place	Total seeds	Germination (percent)	Mortality (percent)	
			Pre-emergence	Post- emergence
Sterile sand	100	45	20	35

however, the development of earlier symptoms is more prominent on older leaves of the plant.

If infected seeds were planted, pre and post emergence damping off might occur. Dark brown sunken lesions developed on cotyledons of emerging seedlings. These lesions extended to hypocotyl and radicle. Under humid conditions cotyledons became water soaked and fell off. The fungus could enter from cotyledons into the stem where small deep seated cankers are formed and may kill the young plant Intermittent rains at frequent intervals favour the epidemic development of the disease. Abundant infections occur at optimum temperature between 17-24^{0}C and relative humidity 100 per cent. Fig. 1 is showing Disease cycle of *Colletotrichum capsici* f. sp. *cyamopsicola.*

Discussion

Survival of the fungus was examined by inserting the parts of leaves packed in paper envelop and buried at varied depth *viz.* 5,10,15, 20 and 25 cm in pots filled with sterilised soil. Even after 12 months, the fungus was found viable at 5 and 10 cm depth.Survivability of the pathogen in plant debris at 15, 20, 25 cm depth was lost within 8 months.

Survival and overwintering of the anthracnose fungus has been studied in several crops[4,22]. In soybean anthracnose, these aspects have been well documented[5]. The viability of the pathogen was found limited to 3–5 months in some cases[2,21], while in bean anthracnose similar findings have been reviewed[15]. In chilli, the pathogen was reported to survive in plant debris for up to 10 months, whereas in infected crop debris survival of *C. gloeosporioides* could extend to 8 months[6]. Further observations revealed that initial survivability of 100 percent declined completely within 6 months when debris was placed at 20 and 25 cm depth, and within 7 months when debris was kept at 5, 10 and 15 cm depths[7]. The highest survivability was noted at 5 cm depth. On sorghum seed, the anthracnose pathogen *Colletotrichum graminicola* survived for about 2½ years but failed to persist in soil beyond 9 weeks[11]. *Colletotrichum dematium* and *Colletotrichumlin demuthianum* survived on crop residues (leaves, stems and pods) of *Vigna radiata* mixed in soil .[20]

The site of infection of *C. capsici* f.sp. *cyamopsicola* in seeds was determined through component plating and microscopic observation, which revealed that the fungus was internally seed-borne and localized in the seed coat. It was further noted that the pathogen could occupy the inner layers of *C. graminicola*[14] and *C. truncata*[8] seed coat. Extra- and intra-embryonal infections have also been reported in certain species. *Colletotrichum* species have been found both externally and internally in seeds of various crops including cowpea, horse gram, kenaf, rice, soybean, black gram, maize, sorghum, and linseed. Several species such as *C. dematium, C. graminicola, C. gloeosporioides, C. lindemuthianum, C. lini and C. acutatum* were associated with these hosts[9]. In soybean seed*Colletotrichum dematiumf.sp. truncatum*, the fungus *Mycelium* was observed to colonize all three layers of the seed coat, with acervuli particularly present in the palisade layer[12].

Seed to plant transmission was studied by sand method. Twenty percent pre-emergence and 35 post-emergence mortalities were recorded.Seeds of inoculated pods were collected and plated on potato sucrose agar medium as such and after surface sterilization. It was found that *C. capsici* f.sp. *cyamopsicola*, *Alternaria cyamopsidis* and *Aspergillus* spp. were found predominantly on the sterilized and unsterilized seed.

The knowledge of disease cycle of a fungus is very essential to locate the weakest link which may be attacked were better management. Disease cycle of *C. capsici* f.sp. *cyamopsicola* has not been fully understood. Several studies have been undertaken in this regard. Seed borne nature of the fungus has been established. Seed to plant transmission was studied by sand method. The seed and seedling infections were confirmed due to *C. capsici* f.sp. *cyamopsicola* by the isolations. Seed transmission of *C. capsici* has also been recorded in urid[17], soybean[1] and sorghum[13]respectively.

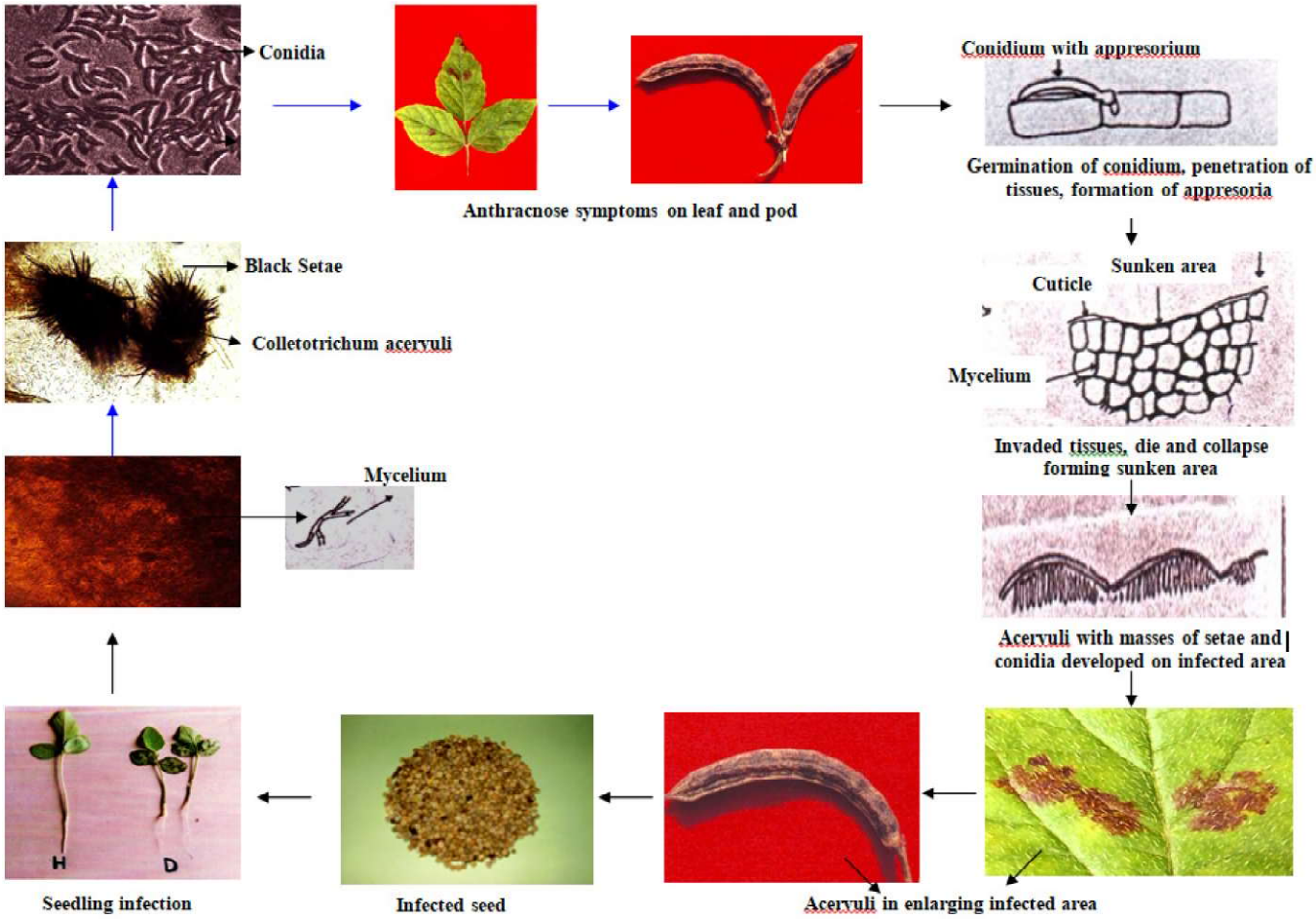

Fig. 1: Disease cycle of anthracnose of guar caused by *Colletotrichum capsici* f.sp. *cyamopsicola*

Site of infection of *C. capsici* f.sp. *cyamopsicola* was observed by seed component plating method. *C. capsici* f.sp. *cyamopsicola* was observed to be internal nature and extra embryonal nature of *C. capsici* f.sp. *cyamopsicola*[16]. Plant to seed transmission was studied by pod inoculation technique. Survival of fungus was tested by placing the anthracnose infected portions of leaves stored in paper envelop and buried at different depths *viz.* 5,10,15,20 and 25 cm in pots filled with sterilized soil. Fungus was viable even after 12 months at 5 and 10 cm depth. No reports are available on the survival of *C. capsici* f.sp. *cyamopsicola.*

Conclusion

In crop debris the fungus remained viable even after 12 months at 5 and 10 cm depths. The fungus was located in the inner layer of seed coat. Twenty per cent pre-emergence mortality and 35 per cent post-emergence mortality were recorded in the sand method.

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Variation in cone morphology, seed traits and germination behaviour of *Cedrus deodara* a study of Bhaderwah forest division (Jammu and Kashmir), India

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ABSTRACT

Cedrus deodara G. Don, a key conifer of the Western Himalayas, plays a vital ecological and silvicultural role in montane forest ecosystems. This study investigated altitudinal variation in cone morphology, seed traits, and germination behavior across five elevations (1421–2747 m) in the Bhaderwah Forest Range, Jammu and Kashmir, India. Significant variation was observed among seed sources in cone length (8.98–11.82 cm), cone weight (105.4–249 g), and seed number per cone. Seed traits such as length (1.41–1.61 cm), width (5.98–7.07 mm), and thickness (2.87–3.63 mm) also varied with altitude. Germination experiments revealed that seeds from mid-altitudes, particularly 2204 m (A3), exhibited the highest germination percentage (96%), shortest mean germination time (11.89 days), and highest germination index (9.30). Seedling vigor, assessed through radicle and plumule length and vigor index, was also highest at A3. In contrast, seeds from the lowest altitude (A1, 1421 m) showed the poorest performance. The findings suggest that mid-altitude sources offer the most promising seed material for reforestation and afforestation efforts in the region. Altitude-driven variation in reproductive and physiological traits underscores the need for site-specific seed sourcing to enhance forest regeneration success.

Figure : 00 References : 18 Tables : 05
KEY WORDS : Cone, Germination, Morphology, Seed, Traits, Western Himalayas

Introduction

The selection of superior seed sources is a critical component of tree improvement programs and ecological restoration efforts, particularly for high-value coniferous species like *Cedrus deodara* (Roxb.) G. Don. Commonly known as Deodar cedar, this species is native to the Western Himalayas and is ecologically, economically, and culturally significant. It forms extensive forest stands and is valued for its durable timber, medicinal properties, and ornamental use. However, successful propagation and regeneration of *Cedrus deodara* depend largely on the availability of genetically and physiologically superior seeds.

Altitudinal gradients profoundly influence the phenotypic and physiological traits of forest trees. Variations in temperature, moisture, soil characteristics, and photoperiod across elevations can significantly affect cone morphology, seed development, germination behavior, and seedling vigor. Understanding these variations is vital for identifying optimal seed sources that ensure better survival, growth, and adaptability in plantation and restoration projects.

Previous studies have documented that seeds collected from mid-altitudinal zones often exhibit better germination and seedling performance due to moderate environmental conditions that favor seed maturation and physiological development. However, region-specific data for *Cedrus deodara* remain limited, particularly in

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TABLE-1 : Description of the study site. Location- Bhaderwah Forest Division

S.No	Altitude (m)	Latitude N	Longitude E
1.	A1=1421	33.056	75.60
2.	A2=1800	32.93	75.70
3.	A3=2204	32.91	75.72
4.	A4=2490	32.90	75.73
5.	A5=2747	32.91	75.75

the Bhaderwah Forest Range of Jammu and Kashmir, India.

The present study aims to evaluate the altitudinal variation in cone morphology, seed traits, and germination parameters of *Cedrus deodara* across five distinct elevations ranging from 1421 m to 2747 m. By assessing these variations, the study seeks to identify the most suitable altitudinal source(s) for quality seed procurement to support afforestation, reforestation, and conservation initiatives in the Western Himalayas.

Methodology

Study area and seed source selection

The study was conducted in the Bhaderwah Forest Range, District Doda, Jammu and Kashmir, India. Five altitudinal zones were selected for sampling, ranging from 1421 m (A1) to 2747 m (A5) above sea level. The geographical coordinates and average climatic conditions of the sites were obtained from the Indian Meteorological Department, Bhaderwah Station. These sites were chosen to represent a wide altitudinal gradient for assessing phenotypic and physiological variations in

TABLE-2 : Altitudinal variation with respect to cone characteristics of *Cedrus deodara*

Altitude	Cone Length (Cm)	Cone Weight (Gm)	Cone Diameter (Cm)	L/D Ratio	Scales/ Cone	No. Of Seeds/ Cone
A1	8.98±0.92	105.4±6.39	5.52±0.66	1.62	135±5.52	138±15.6
A2	11.71±0.71	225±10.24	6.03±0.61	1.85	135±6.88	175±21.6
A3	11.82±0.82	249±10.67	7.70±1.07	1.53	138±5.56	170±16.9
A4	11.11±0.11	160.8±8.55	6.15±0.52	1.80	137±4.52	142±11.2
A5	9.73±0.34	172.2±4.90	6.84±0.29	1.41	119±8.84	140±13.3
Mean	10.67	182.48	6.45	1.64	132.8	153.0
Coefficient of variance	11.80	30.94	13.08	11.19	5.89	11.73
C.D	**0.10**	**2.43**	**0.09**	**0.03**	**2.51**	**2.09**

TABLE-3 : Altitudinal variation with respect to seed characteristics of *Cedrus deodara.*

Altitude	Seed Length (Cm)	Seed Width (Mm)	Seed Thickness (Mm)	Seed Length With Wings (Cm)	L/W	No. Of Seeds In 100gm	Seed M.C%
A1	1.41±0.057	5.98±0.46	2.87±0.25	3.43±0.49	0.23	390±11.6	19.29±0.074
A2	1.52±0.067	6.59±0.68	3.59±0.49	3.43±0.49	0.23	416±14.6	19.03±0.224
A3	1.61±0.015	7.07±1.16	3.63±0.53	4.46±1.08	0.22	402±6.9	19.23±0.004
A4	1.47±0.117	6.22±0.62	3.51±0.41	3.92±0.54	0.23	409±7.6	19.29±0.074
A5	1.46±0.107	6.44±0.84	3.12±0.72	3.86±0.48	0.22	397±11.6	19.23±0.004
Mean	1.494	6.46	3.34	3.82	0.226	402.8	19.21
Coefficient of variance	5.07	6.37	9.97	11.15	2.42	2.52	0.56
C.D	**0.03**	**0.05**	**0.01**	**0.01**	**0.002**	**1.93**	**0.11**

Cedrus deodara.

Mature cones were collected during October from each of the five altitudinal sources. Ten cones were randomly harvested from each of five phenotypically superior trees per source (totaling 50 cones per altitude). The cones were collected by climbing the tree and were carried in cotton bags and transported to Faculty for evaluation.

In the laboratory, cones were air-dried for 7–10 days to induce dehiscence. Morphological parameters such as cone length, diameter, weight, number of scales per cone, and the number of sound and unsound seeds per cone were recorded using digital calipers and electronic balances.

Seed Trait Analysis

After extraction, seeds were stored at room temperature until analysis. Seed morphology was studied using twenty five (25) seeds per source in four replicates. Measured traits included seed length (with and without wings), seed width, and seed thickness using a digital vernier caliper. Seed weight and number of seeds per 100 g were also determined. Seed moisture content was calculated by oven-drying 20 g of seeds at 104°C for 24 hours, using the following formula:

Moisture Content = wet weight – dry weight */* wet weight

Germination Experiment

Seeds were soaked in distilled water for 24 hours before germination tests. Germination was conducted in 9 cm Petri dishes lined with Whatman No. 1 filter paper, moistened with distilled water, and incubated in a germinator at constant temperatures of 15°C and 20°C. Each treatment included four replicates of 25 seeds.

Germinated seeds (with radicle emergence e" 2 mm) were recorded daily for 21 days. Filter papers were replaced weekly to prevent fungal growth. Germination percentage (GP), mean germination time (MGT), and germination index (GI) were calculated using the following formulas:

Mean germination time = © (ni * ti)/N

where ni = the number of seeds that germinated (2 mm radicle length) in a day.

ti= time (hours).

N = the total number of seeds that germinated in the period of experiments.

The germination index (GI) was calculated by following :-

¢' Germination Index = © (Gt/Dt),

TABLE-4 : Altitudinal Variation with Respect to Germination (%), Mean Germination Time, and Germination Index *of Cedrus deodara.*

Altitude	Germination %	Mean Germination Time	Germination Index
A1	84±3.2	12.50±1.16	7.71±0.45
A2	92±2.8	12.62±1.10	8.24±0.42
A3	96±2.5	11.89±1.13	9.30±0.47
A4	88±3.0	13.34±1.09	7.11±0.41
A5	64±3.5	16.05±1.15	4.15±0.38
Mean	84.8	13.28	7.30
Coefficient of variance	14.69	12.29	26.53
C.D	**2.55**	**1.52**	**0.93**

where Gt = the number of germinated seeds at the end of the germination period.

Dt = the total days for germination.

Seedling Vigour Assessment

Seedling vigor was evaluated by measuring radicle and plumule lengths of germinated seedlings after 21 days. The seed vigor index (SVI) was calculated by multiplying the sum of radicle and plumule lengths with the germination percentage:

SVI = (Radicle length + Plumule length) * Germination%

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) to assess the significance of differences among altitudinal sources. The normality of residuals was tested using the Shapiro-Wilk test, homogeneity of variance with Bartlett's test, and independence of errors with the Durbin-Watson test. Mean separation was performed using Fisher's Least Significant Difference (LSD) test at a 5% significance level.

Results

Cone Morphology

Significant differences ($p < 0.05$) were observed in cone morphology of *Cedrus deodara* across altitudinal gradients (Table 2). Cones from the mid-altitude site A3 (2204 m) recorded the highest mean cone length (11.82 ± 0.82 cm), diameter (7.70 ± 1.07 cm), and weight (249 ± 10.67 g), while the lowest values were observed at the high-altitude site A5 (2747 m). These findings are consistent with earlier works[4], who also reported superior cone development in mid-elevation populations of *Abies pindrow*, attributing it to more favorable temperature and nutrient availability.

The number of scales per cone peaked at A3 (138), whereas the lowest was at A5 (119). A2 showed the highest number of seeds per cone (175 ± 21.6), while A1 had the lowest (138 ± 15.6). Similar to previous work[15], our results suggest that mid-altitudes provide optimal microclimatic conditions for cone and seed development in Himalayan conifers.

Seed Morphology

Seed morphological traits also varied significantly with altitude (Table 3). Seeds from A3 had the greatest average length (1.61 ± 0.015 cm), width (7.07 ± 1.16 mm), thickness (3.63 ± 0.53 mm), and seed length with wings (4.46 ± 1.08 cm). A1 recorded the lowest values for most traits. These results align with findings[11], who noted that seeds from higher altitudes were generally smaller, possibly due to lower temperatures affecting seed maturation.

Seed weight was highest at A2, indicating that heavier seeds may originate from intermediate

TABLE-5 : Radicle, Plumule Length (cm), and Seed Vigour Index of Seedlings.

Altitude	Radicle Length (cm)	Plumule Length (cm)	Seed Vigour Index
A1	3.3	7.6	915.6
A2	4.1	10.4	1334
A3	4.9	13.3	1747
A4	3.8	9.2	1144
A5	3.4	7.7	710
Mean	3.9	9.64	1170.12
Coefficient of variance	16.52	24.38	34.10
C.D	**0.01**	**0.03**	**98.50**

elevations with optimal nutrient accumulation. This observation parallels the earlier results[18], who found a positive correlation between seed weight and germination success in Himalayan conifers.

Germination Performance

Germination parameters varied significantly ($p < 0.001$) among sources (Table 4). A3 exhibited the highest germination percentage (96 ± 2.5%), the shortest mean germination time (11.89 ± 1.13 days), and the highest germination index (9.30 ± 0.47). These findings mirror those of previous ihnvestigators[8,17], who observed that seeds from mid-altitudes germinate faster and more uniformly, likely due to balanced dormancy and optimal physiological maturity.

Seedling Vigour

Seedling vigor, a key indicator of early establishment success, also varied with altitude (Table 5). A3 recorded the highest radicle length (4.9 cm), plumule length (13.3 cm), and seed vigor index (SVI: 1747), followed by A2 (SVI: 1334). A5 again ranked lowest (SVI: 710). These trends support the conclusions[3,12], who found that seedling vigor is highest in populations originating from ecologically moderate elevations, due to superior seed reserves and metabolic activity.

Statistical Analysis

The Shapiro-Wilk test confirmed normal distribution of residuals ($p = 0.221$), while the Other test indicated independence ($p = 0.065$). Bartlett's test detected non-homogeneous variance ($p = 0.006$), which justified the use of LSD-Fischer post hoc analysis. The critical difference (CD) for germination percentage was 7.37, confirming statistically significant differences among altitudinal sources.

Discussion

This study revealed considerable altitudinal variation in cone morphology, seed traits, germination behavior, and seedling vigor of *Cedrus deodara* across five elevations in the Bhaderwah Forest Range. The findings indicate that cone and seed characteristics, as well as germination and seedling performance, were significantly influenced by elevation, suggesting environmental adaptations or ecotypic differentiation among the sampled populations.

Among all sources, the mid-altitudinal site A3 consistently outperformed the others in most parameters. A3 recorded the highest cone length (11.82 ± 0.62 cm), cone diameter (7.70 ± 1.07 cm), and cone weight (249.1 ± 10.67 g), along with a high number of seeds per cone (170 ± 16.9). These results are consistent with earlier ones[4], who also reported superior cone development in mid-elevation populations of *Abies pindrow,* attributing it to favorable temperature and nutrient availability. In contrast, the lowest values for these cone parameters were observed at the highest altitude, A5, where the cone weight was only 172.2 ±

4.90 g, and the number of seeds per cone was significantly reduced (140 ± 13.3). The lower productivity at A5 may be attributed to environmental stressors such as lower temperatures and shorter growing seasons, which can negatively impact cone development and seed filling.

Seed morphometric traits also varied significantly with altitude. Seeds from A3 showed maximum values for seed length (1.61 ± 0.015 cm), seed width (7.07 ± 1.16 mm) and seed thickness (3.65 ± 0.53 mm), while A1 recorded the lowest values in all three dimensions. These results align with earlier finding[11], that seeds from higher altitudes were generally smaller, possibly due to lower temperatures affecting seed maturation. Seed length with wings was also highest at A3 (4.46 ± 1.08 cm), suggesting better potential for wind dispersal. Although seed moisture content was relatively stable across all sources (around 19.2%), the number of seeds per 100g was again highest at A2 (416 ± 14.6). This indicates denser, better-formed seeds at mid-altitudes. This observation parallels the results[18], that a positive correlation between seed weight and germination success in Himalayan conifers.

Germination parameters also varied significantly among sources. The highest germination percentage was recorded at A3 (96 ± 2.5%), followed by A2 (92 ± 2.8%), whereas A5 displayed the lowest germination (64 ± 3.5%). Similarly, the germination index, which reflects both the speed and uniformity of germination, was highest at A3 (9.30 ± 0.47) and lowest at A5 (4.15 ± 0.38). These findings mirror those[8,17] which observed that seeds from mid-altitudes germinate faster and more uniformly, likely due to balanced dormancy and optimal physiological maturity. The mean germination time was shortest at A3 (11.89 ± 1.13 days) and longest at A5 (16.05 ± 1.15 days), suggesting delayed or staggered germination under harsh conditions at higher elevations.

These trends extended into early seedling development. The A3 provenance yielded seedlings with the greatest radicle length (4.9 cm), plumule length (13.3 cm), and the highest seed vigor index (1747). This reflects a strong correlation between seed quality and early growth potential. This is consistent with[3,12], which found that seedling vigor is highest in populations originating from ecologically moderate elevations, due to superior seed reserves and metabolic activity. In comparison, A5 again showed reduced performance, with a seed vigor index of only 710. This pattern indicates that environmental conditions at mid-elevations not only favor the production of larger, heavier cones and seeds but also result in improved germination success and more vigorous seedling growth.

Taken together, the data demonstrate that *Cedrus deodara* populations at mid-altitudes (particularly A2 and A3) are morphologically and physiologically better adapted to local conditions, resulting in enhanced reproductive success and seedling establishment. Conversely, populations at the highest elevation (A5) exhibit reduced performance across all measured parameters, likely due to harsher climatic conditions and limited resource availability. These findings have important implications for conservation and afforestation programs, suggesting that mid-altitude provenances may be the most suitable sources for seed collection and nursery propagation efforts aimed at restoring or expanding *Cedrus deodara* populations.

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Perspective for sustainable future in urban landscape of Delhi, India

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ABSTRACT

The air quality of Delhi has been alarming over the past few years and as per the data base of World Health Organization, the city has exceeded the limits of clean air guidelines. United Nations Sustainable Development Goal 11 promotes to make cities inclusive, safe, resilient and sustainable. This short note attempts to indicate the perspective of sustainable future in the urban land scape of Delhi. It is well recognized fact that urban areas need to conserve and promote biodiversity for enhancing the urban resilience. Environment awareness programmes where residents take part voluntarily is the need of hour. Moreover, effective environment awareness would help individuals adopt green habits so that the future generation will have greener environment.

Figure : 01 References : 28 Table : 01
KEY WORDS : Biodiversity conservation, Delhi, Sustainable future, Urban land scape

Introduction

Urbanization is one of the major factors leading to land use change of a region which has led to the development of a large number of cities[7]. The proportion of the global human population inhabiting urban areas has rapidly increased over time, rising from 2% in 1800 to 47% in 2000 [8, 16,17].The human population is expected to rise to be about 9.3 billion in 2050 and it is predicted that 66% of people would live in urban areas[8,17]. Urban areas have been the most severely impacted by the increase in land degradation for the past two decades[22]. Factors like habitat loss, competition from introduced species, human demand for certain species and products, and rapid environmental changes are contributing to biodiversity loss[10]. Promoting and preserving biodiversity within urban green-space is one way to decelerate the rapid rate of biodiversity loss as the world becomes more urbanized[1].

United Nations Sustainable Development Goal 11 promotes inclusive, safe, resilient, and sustainable cities. Resilient and sustainable urban development depends on policies and practices that prioritize access to basic services, affordable housing, efficient transportation and green spaces for all[27]. There is growing recognition of urban areas to conserve and promote biodiversity and parks, which are biodiversity hotspots in the cityscape[20]. This short note attempts to indicate the perspective of sustainable future in the urban landscape of Delhi.

Delhi, the capital of India and one of the most densely populated cities in the world covers an area of 1,483 sq. km with forest cover[14] of 195.28sq.km. As per Census of India[5], 2011 the city is having population of 1.6 crore. It is geographically located in the coordinates of 76.84°E, 28.41°N, by 77.35°E, 28.88° N; and characterized by hot & dry summers and cold winters[15]. Rainfall is dominated by monsoonal weather pattern, with

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TABLE- 1 : Urban biodiversity parks in Delhi

S.No.	Name of the biodiversity park	Area of the park	Provisions
1.	Yamuna Biodiversity Park	184 ha	✓ Eco-system services ✓ Eco-tourism ✓ Research opportunity ✓ Environment Education
2.	Aravalli Biodiversity Park	280 ha	
3.	Northern Ridge	87 ha	
4.	Tilpath valley Biodiversity Park	70 ha	
5.	NeelaHauz Biodiversity Park	3 ha	
6.	Tughlaqabad Biodiversity Park	130 ha	
7.	Kalindi Biodiversity Park	167 ha	

Source: Delhi Development Authority, https://dda.gov.in/biodiversity-parks

maximum rainfall occurring from June to September. Delhi has presence of threatened spp. viz. Hog deer (Endangered), Striped Hyena (Near Threatened), Leopard (Vulnerable), Sambar (Vulnerable)[18]. More than 400 bird species have been recorded from Delhi and adjacent areas, including resident and migratory species; of these, nearly 30 species were identified as globally threatened [4]. Rapid urbanization in the city has resulted in increased pollution, decreased water percolation into ground, lesser green cover and urban heat island phenomenon[15].

Biodiversity conservation

Conservation approaches for urban biodiversity depends upon the people who live in the cities and recognition of their experiences[21]. A study[25] identified the conservation actions undertaken by urban environmental managers *viz.* managing threats (*eg.* weeding, pest control, fencing), restoring grasslands, native species, adding structures like nesting hollows, governance, and coordination/outreach through community science program. The creation of biodiversity parks is regarded as one of the most innovative techniques to address the issue of biodiversity loss [22]. As on date, Delhi has seven urban biodiversity parks (Fig. 1 and Table-1). These parks play important role in achieving Target 3 of Kunming-Montreal Global Biodiversity Framework[9] which calls for the conservation of at least 30% of terrestrial, inland water, coastal and marine areas globally through "well-connected systems of protected areas and other effective area-based conservation measures, recognizing Indigenous and traditional territories". Also conservation of these urban green spaces is most beneficial for urban heat mitigation[11].

Clean Air

The air quality of Delhi has been alarming over the past years and as per the database of World Health Organization the city has exceeded the limits of clean air guidelines. The worst air quality has been responsible for 10,000 premature deaths per year in Delhi [6]. To improve the air quality in Delhi, National Clean Air programme was launched in year 2019 in 130 cities (non-attainment cities and Million Plus Cities) including Delhi. It enforces stringent adherence to set emission regulations, acknowledging that various sources like industries, vehicles, construction, open biomass burning and dust from roads are responsible for air pollution in Delhi [13, 26]. To monitor the implementation of programme, cities have been mandated to submit quarterly progress report on 'PRANA' portal. Delhi's action plan include mechanical road sweeping, water sprinkling using treated sewage water, solid waste management, Construction & Demolition waste management *etc.*[23]. It has been reported[12] that the air pollution's cross-sectoral and cross-regional nature calls for cross-state/cross-departmental coordination, thereby an 'airshed management approach' to be considered to compliment city's efforts with the regional efforts.

Further Ministry of Environment, Forest and Climate change is implementing *Nagar Van Yojana* which envisages developing *nagar vans* [urban forests] in order to significantly enhance ecological benefits in urban and peri-urban areas[19]. It involves local communities, NGOs, local bodies, educational institutions to conduct activities like plantation of native shrubs, tree species, soil - moisture conservation measures and fencing. Local

Fig. 1 : Wetland inside Yamuna Biodiversity Park of Delhi which provides habitat for various migratory birds

municipalities and urban local bodies may also focus on the legal provisions for biodiversity and mobilize resources by leveraging corporate social responsibility funding[24].

Circular economy

Circular economy is a useful tool for enhancing the world's sustainability wherein all the products are recycled and no waste is sent to landfills, incineration or other disposal methods. Circular economy development model provides environmental benefits by demonstrating waste management benefits[28]. For example, ash which is generated from coal or lignite based thermal power plants have been mandated to be utilised for eco-friendly purposes like Fly ash based products *viz.* bricks, blocks, tiles, fibre cement sheets, pipes, boards, panels; cement manufacturing, ready mix concrete *etc*[3] . Therefore, fly ash being a waste product transforms into a valuable resource and promotes circular economy in sectors like infrastructure. Additionally, Plastic Waste Management Rules, 2016, and the Plastic Waste Management Amendment Rules, 2021, enforces Extended Producer Responsibility (EPR) for plastic packaging, mandating recycling and reuse[19]. Through the EPR regulations, the producer, importer or brand owner who introduces the product in the market are obligated under law for its environmentally sound management after end-of-life of such products.

Conclusion

A report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services revealed that almost half (47%) of threatened terrestrial mammals, excluding bats, and about 23% of threatened birds may have already been negatively affected by climate change in atleast part of their distribution[10]. It is very crucial to understand, recognize the importance of urban biodiversity and preserve the same as the quality of life, well being of urban residents is linked to it. Environment awareness programmes where residents take part voluntarily is the need of hour. *Great Backyard Bird Count-India* wherein over 100 birders in Delhi explored bird diversity in their neighbourhood highlighted the importance of scientific documentation and community engagement[2]. Such scientific knowledge can be used in urban planning and management thereby

maintaining the ecological services to secure sustainable future. Moreover, effective environment awareness will help individuals adopt green habits so that our future generation have greener environment.

Conflict of interest/Competing interest

The authors have declared that there is no conflict of interest. Perspective for sustainable future in urban landscape of Delhi, India

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Geospatial Analysis of Rainfall Variability in the Trans-Saryu Region in Uttar Pradesh, India

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ABSTRACT

This study investigates the spatial and temporal variability of rainfall in the Trans-Saryu Region of Eastern Uttar Pradesh, India, using satellite-derived rainfall data from the Centre for Hydrometeorology and Remote Sensing (CHRS) and the Inverse Distance Weighting (IDW) interpolation technique. The analysis reveals a pronounced west-to-east gradient in rainfall distribution, with western districts experiencing lower rainfall compared to eastern districts. The change detection analysis highlights a dual climatic challenge: progressive aridity in the west and rainfall intensification in the east. The findings emphasize the need for area-specific adaptation measures, such as groundwater recharge and drought-proofing in the west, flood management in the east, and integrated water resource management in the central belt.

Figures : 03 References : 12 Table : 01

KEY WORDS : CHRS data, IDW interpolation, Rainfall variability, Spatial analysis, Temporal analysis, Trans-Saryu region

Introduction

Rainfall plays a pivotal role in shaping hydrological processes and strongly influences water resource availability, agriculture, and disaster management. Its distribution, however, is far from uniform; it exhibits pronounced spatial and temporal variability, particularly in the tarai regions where diverse topographical features and climatic influences interact.[4] Understanding rainfall variability in such landscapes is essential not only for sustainable agricultural planning but also for effective water resource management and flood risk reduction[5,7,11]. The Trans-Saryu region, characterized by its relatively flat terrain yet subjected to varying climatic influences, presents a unique case for examining rainfall distribution patterns. A systematic analysis of rainfall variability in this region can provide valuable insights to support sustainable development and climate resilience initiatives.

The growing reliance on satellite-based precipitation products has transformed rainfall research, particularly in areas where ground-based monitoring networks are inconsistent[6]. The Centre for Hydrometeorology and Remote Sensing (CHRS) at the University of California, Irvine (https://chrsdata.eng.uci.edu/) provides high-resolution product, with a spatial resolution of 0.04° × 0.04° (approximately 4 km × 4 km), delivers near-real-time global precipitation estimates between the latitudes 60°S to 60°N. Such coverage makes it particularly advantageous for regions like the Trans-Saryu, where dense rain-gauge networks

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TABLE-1 : District-wise Change in Average Annual Rainfall (2004–2024)

District	Change in Rainfall (mm)	Category(Key Concern)
Bahraich	–310 to –221	Severe Decline(Increasing aridity, risk of drought)
Shrawasti	–310 to –221	Severe Decline(Sharp reduction in rainfall, risk of drought)
Gonda	–172 to –124	Moderate Decline(Declining rainfall, reduced soil moisture)
Balrampur	–124 to –81	Moderate Decline(Transition zone, drought risks emerging)
Basti	–40 to –3	Slight Decline(Near stable, vulnerable to seasonal variability)
Sant Kabir Nagar	–40 to –3	Slight Decline(Stable but sensitive to rainfall fluctuations)
Siddharth Nagar	–3 to +40	Slight Increase(Transitional zone, mixed drought/flood risks)
Maharajganj	–40 to +40	Stable(Minor changes)
Kushinagar	+122 to +214	Significant Increase(Intensified rainfall, inundation risk)
Gorakhpur	+122 to +214	Significant Increase(Sharp rise in rainfall, increased flood risks)
Deoria	+122 to +214	Significant Increase(Intensified rainfall, flood stress)

Source: Computed from satellite data of 2004-2024

are absent.

To translate the estimates into meaningful spatial patterns, interpolation techniques are often used in hydrology, climatology, and environmental sciences. These methods create continuous surfaces from discrete data points, allowing researchers to recognize heterogeneity and regional differences in rainfall. Among these, the Inverse Distance Weighting (IDW) technique has become a popular approach because of its simplicity and effectiveness.[7] Studies have indicated that the accuracy of IDW depends on parameters such as the radius of influence and the power value used in distance weighting. However, the performance of IDW is not universal; it is influenced by grid resolution and the underlying physiographic characteristics of the study area. In mountainous catchments, for instance, interpolation accuracy is more sensitive to power values than grid size.[3] Recent advancements, such as dual IDW methods, further refine its application by incorporating data-to-data correlations and locally varying parameters, thereby reducing error and better capturing localized rainfall dynamics.[8]

Considering these developments, assessing rainfall variability in the Trans-Saryu region using CHRS satellite data and the IDW interpolation method provides a solid framework for filling gaps in observational networks. This approach ensures accurate spatial rainfall mapping, supporting water management, agricultural decisions, and disaster preparedness in this climate-sensitive area[1].

Aim of the Study

The study aims to investigate the spatial and temporal variability of rainfall in the Trans-Saryu region to generate reliable rainfall distribution patterns, compare historical and recent trends, and provide insights for sustainable resource management and climate adaptation strategies.Despite the availability of rainfall studies in Uttar Pradesh, limited research focuses on the Trans-Saryu region using high-resolution satellite data. Ground station scarcity restricts spatial analysis. This study bridges the gap by employing CHRS data

and IDW interpolation in ArcGIS to generate reliable rainfall variability patterns for regional planning.

Objectives of the Study

1. To analyse spatial and temporal variations in rainfall across the Trans-Saryu region using CHRS satellite-based datasets.
2. To generate averaged rainfall maps for the baseline period (2004–2009) and the recent period (2020–2024) to minimize annual variability and enable meaningful comparison.
3. To apply the Inverse Distance Weighting (IDW) interpolation technique in ArcGIS for developing continuous spatial surfaces of rainfall distribution.
4. To compare past and recent rainfall scenarios to identify significant trends, shifts, or anomalies in rainfall patterns.

Study Area -Trans-Saryu Region

The Trans-Saryu region mainly covers 11 districts of the Northern East Gangetic Plain. Its topography is very flat and low-lying, shaped by sediment from the Saryu, Ghaghara, and Ganges River systems over thousands of years. The elevation varies little, usually between 50 and 100 meters above sea level, creating a gentle slope toward the southeast[9]. The region's water system is dominated by the Saryu River and its network of tributaries and distributaries, making the area prone to flooding during the monsoon season. The river channels often change course over time, leaving behind old oxbow lakes and tals (seasonal wetlands), which are common features of the terrain[10].

The Trans-Saryu region experiences a monsoon-influenced humid subtropical climate. This region experiences considerable spatial variability in rainfall distribution, making it an ideal case study for understanding complex precipitation patterns. The annual rainfall averages between 900 mm to 1,200 mm, mostly received from the southwest monsoon. Rainfall is highly variable in its spatial distribution and temporal pattern, often occurring in heavy, intense downpours. This variability is a critical climatic feature, as both excessive rainfalls leading to floods and prolonged breaks leading to drought can occur within the same season. Agricultural practices in the area range from rain-fed cropping systems to irrigated agriculture, further emphasizing the importance of accurate rainfall information for making water management decisions

Methodology

The present study employed a geospatial approach to analyse rainfall variability in the Trans-Saryu region using satellite-based rainfall data and spatial interpolation techniques within the ArcGIS environment. The methodology involved three major steps: data acquisition and preprocessing, temporal aggregation of rainfall records, and geospatial interpolation for spatial pattern analysis.

Data Source and Temporal Framework

Rainfall data were obtained from the online portal of the Centre for Hydrometeorology and Remote Sensing (CHRS) at the University of California, Irvine. The CHRS Precipitation Estimation from Remotely Sensed Information using Artificial Neural Networks (PERSIANN) system provides high-resolution gridded rainfall datasets with a spatial resolution of 0.04° × 0.04° (approximately 4 km). This fine resolution makes it suitable for regional scale hydrological and climatological studies, particularly in data-scarce areas like the Trans-Saryu region.For this study, two distinct temporal windows were selected to enable comparative assessment. The first data set represented the baseline period of 2004–2009, while the second dataset corresponded to the recent period of 2020–2024.To minimize the influence of inter-annual fluctuations and extreme events, average annual rainfall values were computed for each period.

This temporal averaging enabled a more reliable comparison of long-term rainfall conditions, capturing broader climatic trends rather than short-term anomalies.

Data Pre-processing

The satellite-derived rainfall data were downloaded in TIFF format,which is compatible with ArcGIS. Spatial subsets corresponding to the administrative boundaries of the Trans-Saryu region were extracted using a mask layer. The data were then organized into annual and multi-year averages for the two chosen periods (2004–2009 and 2020–2024). This ensured uniformity in the data sets before applying spatial interpolation.

Interpolation Technique

Spatial interpolation was performed using the Inverse Distance Weighting (IDW) technique, which is available in ArcGIS. IDW assumes that the value at an unsampled location could be estimated as a weighted average of nearby sampled values, with closer points exerting greater influence. The method was chosen due to its demonstrated reliability in hydrological applications and its capacity to generate smooth, continuous rainfall surfaces. The interpolation was performed separately for the two temporal data sets, creating gridded rainfall distribution maps for both the baseline and recent periods.

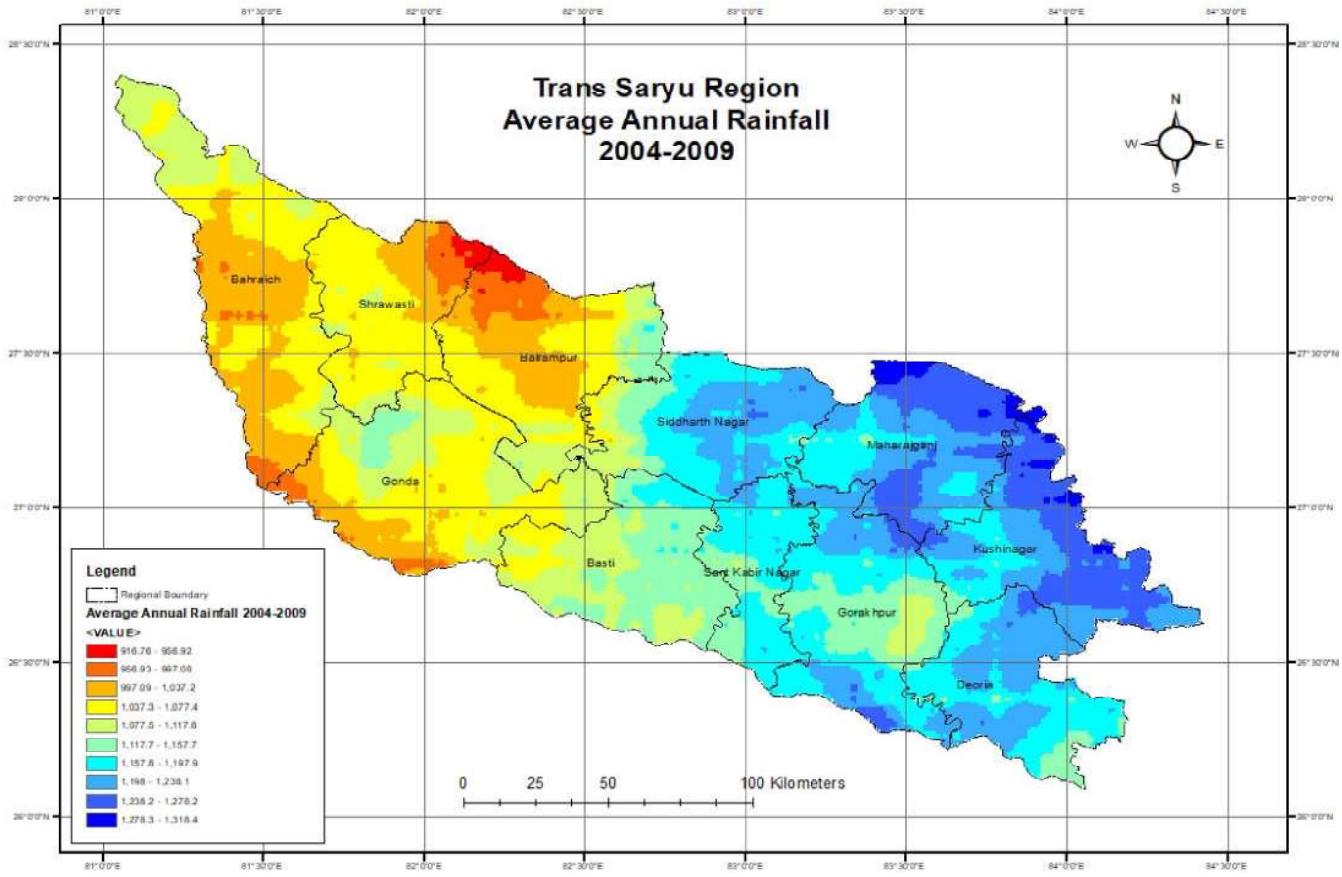

Fig. 1 : Source: Computed from (PERSIANN) Satellite-based Annual Rainfall Data

Comparative Analysis

The interpolated average annual rainfall maps were analysed to identify spatial heterogeneity and shifts in rainfall distribution between the two time frames. By comparing the baseline average (2004–2009) with the recent average (2020–2024), the study was able to highlight long-term changes in rainfall intensity and spatial variability across the Trans-Saryu region. This comparison was critical for assessing emerging rainfall trends and evaluating their implications for agriculture, water management, and climate adaptation strategies in the region.

Results and Discussion

Spatial Variation in Average Annual Rainfall during 2004–2009

The spatial variation of average annual rainfall in the Trans Saryu region during 2004–2009 highlights significant geographical differences across districts (Fig.1). The map depicts a clear west-to-east and northwest-to-southeast transition in rainfall distribution, with districts in the western and northwestern parts receiving comparatively lower rainfall, while the eastern and southeastern districts experience higher precipitation levels. This pattern provides valuable insights into the hydrological regime and agricultural potential of the region.

In the western zone, districts such as Bahraich, Shrawasti, and Gonda recorded relatively lower average annual rainfall. The average annual rainfall amount in these areas generally ranges between 916 mm and 1,037 mm, which places them in the lowest category of rainfall observed across the Trans Saryu region. The brown, orange, and yellow shades dominating these districts on the map confirm a semi-arid tendency when compared to the rest of the region. Such conditions likely create water stress for rainfed agriculture, particularly for crops like paddy that demand high water inputs. Farmers in these areas might have had to rely more heavily on irrigation infrastructure or adapt to less water-intensive crops.

Moving towards the central districts, including Balrampur, Basti, and Sant Kabir Nagar, there is a noticeable transition in rainfall intensity. Balrampur shows some of the highest rainfall concentrations in its northern zone, where the rainfall amount surpasses 1,198 mm annually. This contrasts sharply with its southern counterparts, where rainfall is more moderate. Similarly,

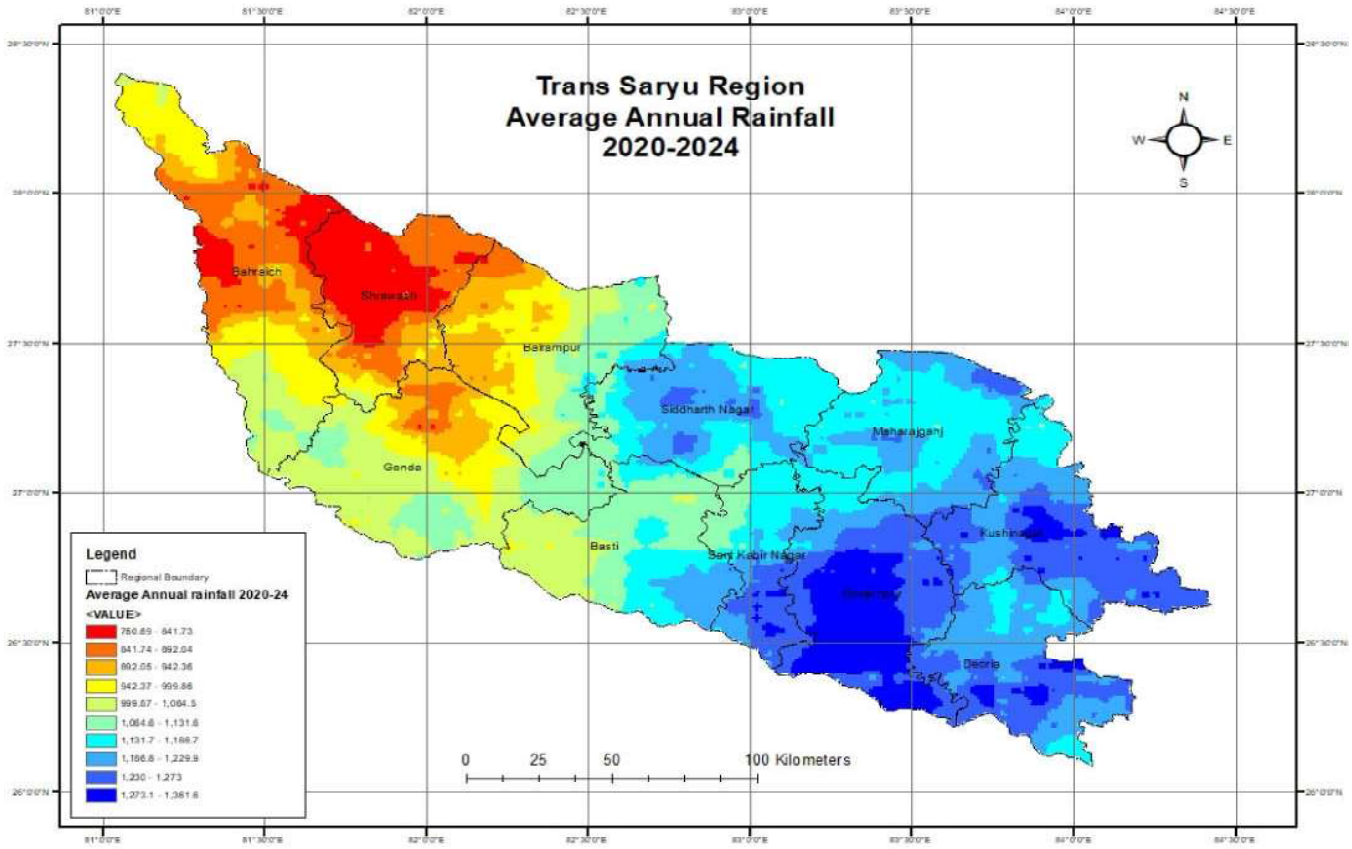

Fig. 2 : Source: Computed from (PERSIANN) Satellite-based Annual Rainfall Data

Basti and Sant Kabir Nagar exhibit moderate levels of rainfall, mostly between 1,077 mm and 1,198 mm. This intermediate rainfall zone acts as a climatic buffer between the drier western districts and the wetter eastern belt. The variation within Balrampur itself suggests localized influences of terrain and monsoon circulation, making it a district of contrasting rainfall patterns.The eastern sector of the Trans Saryu region, comprising Siddharth Nagar, Maharajganj, Kushinagar, Gorakhpur, and Deoria, experiences the highest rainfall during the period. These districts are shaded in light blue to dark blue tones, indicating average rainfall between 1,157 mm and 1,315 mm

Maharajganj and Kushinagar, in particular, stand out as high-rainfall zones, consistently crossing 1,200 mm annually. The abundance of rainfall in these districts supports water-intensive agricultural practices and contributes significantly to groundwater recharge. However, this also brings challenges, such as frequent waterlogging, floods, and soil erosion during heavy monsoon spells. Gorakhpur, located centrally in the eastern part, experiences slightly moderate rainfall compared to Maharajganj and Kushinagar, but still significantly higher than the western districts. Deoria, positioned in the southeast, also records high rainfall levels, reinforcing the eastward gradient of precipitation.

The spatial variation indicates a distinct west-to-east gradient, where rainfall increases progressively across the region. This gradient reflects the influence of the southwest monsoon winds, which weaken as they move westward after entering eastern Uttar Pradesh. The higher rainfall in the eastern districts could also be attributed to proximity to the Himalayan foothills and local climatic conditions that enhance precipitation. Such spatial disparities in rainfall distribution play a critical role in shaping cropping patterns, groundwater availability, and overall vulnerability to climatic risks across the region.

Spatial Variation of Average Annual Rainfall: 2020–2024

The spatial variation of average annual rainfall across the Trans Saryu region during 2020–2024 indicates significant heterogeneity, with a marked west-to-east gradient similar to the earlier period, but with notable shifts in magnitude and spatial concentration (Fig. 2). The figure shows that the western districts, particularly Bahraich, Shrawasti, and parts of Balrampur, experienced considerably lower rainfall levels compared to their eastern counterparts. The average annual rainfall

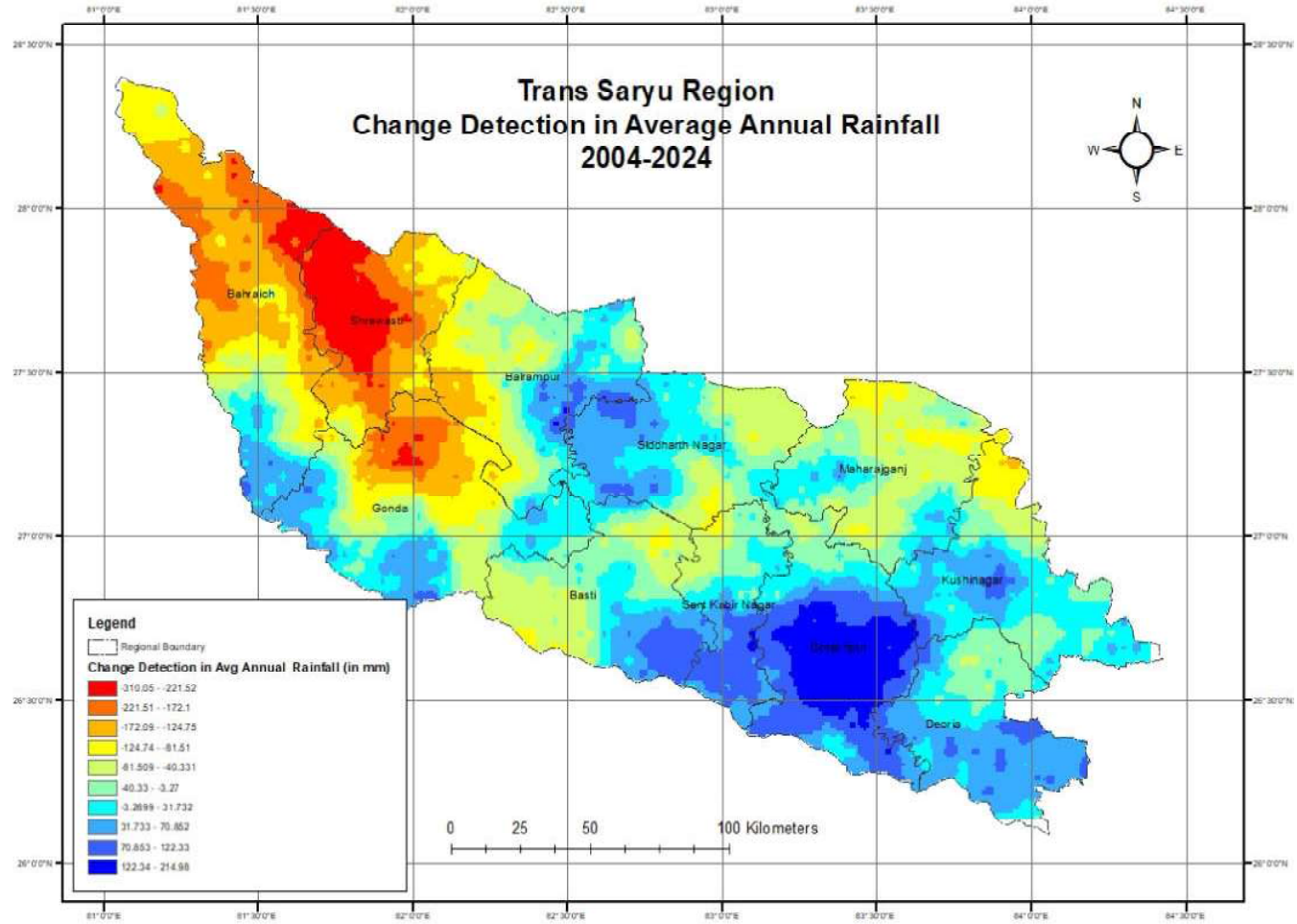

Fig. 3 : Source: Computed from (PERSIANN) Satellite-based Annual Rainfall Data

in these areas now falls within 750–950 mm, representing the lowest category in the region. The red and orange zones dominating this part of the map highlight a worrying trend of increasing dryness in western districts. This suggests a strengthening of the rainfall deficit that was already present in 2004–2009, when these same districts received around 916–1,037 mm of rainfall. The current reduction points toward a potential long-term drying trend in the western Trans Saryu, posing challenges for agriculture and water resource management.

In contrast, central districts such as Gonda, Balrampur, and Basti show intermediate rainfall levels, ranging between 950–1,150 mm. These rainfall amount, although moderate, represent a relative decline in rainfall compared to the earlier period (1,037–1,198 mm).

The yellow and light green shades indicate that these districts form a transitional belt between the low-rainfall western zone and the high-rainfall eastern zone. The overall rainfall decline in these central districts suggests increased vulnerability to rainfall variability, especially for farmers dependent on the kharif paddy crop. Balrampur is particularly notable, as it displayed higher rainfall patches in its northern part during 2004–2009, but in the recent period, these high-rainfall areas have contracted, indicating a shift in rainfall concentration.

The eastern districts, comprising Siddharth Nagar, Maharajganj, Kushinagar, Gorakhpur, and Deoria, continue to experience relatively high rainfall. The rainfall in these districts now ranges between 1,150–1,360 mm, as indicated by the green, blue, and deep blue shades. While this continues the historical trend of high rainfall in the east, a comparison with 2004–2009 reveals subtle changes.

Previously, districts like Maharajganj, Kushinagar, and Deoria consistently received 1,198–1,315 mm of rainfall. Now, rainfall values have expanded slightly upward to 1,273–1,361 mm in some pockets, especially in Deoria and southern Gorakhpur. This indicates localized intensification of rainfall events, which may be linked to stronger monsoon systems or climate variability. While beneficial for water availability and irrigation potential, such intensification increases the risks of flooding, waterlogging, and soil erosion.

Absolute Change Detection in Average Annual Rainfall

The spatial change detection of average annual rainfall in the Trans-Saryu region between 2004 and 2024 reveals striking patterns of variability, with distinct west–east contrasts. Figure 3 highlights areas of decline in rainfall in the western districts and increases in the eastern and southern districts, pointing to a shift in the hydrological regime that has significant implications for water management and agriculture

In the western districts such as Bahraich and Shrawasti, the figure shows the most severe negative change, with rainfall reductions ranging between –310 mm and –221 mm (Table-1). These areas fall under the high-decline category, signalling the onset of progressive aridity. Such a decline will likely intensify water stress, reduce soil moisture availability, and adversely affect rainfed agriculture. Similar but slightly lower declines are observed in Gonda, where reductions range from –172 mm to –124 mm, placing it in the moderate-decline zone. These trends confirm a westward shift toward drier conditions, requiring urgent adaptation measures such as drought-resistant cropping systems and irrigation expansion.

In contrast, the eastern districts present an opposite trend. Deoria, Gorakhpur, and Kushinagar show substantial positive changes, with increases of +122 mm to +214 mm. Gorakhpur, in particular, demonstrates significant intensification of rainfall, which could aggravate the district's existing flood and waterlogging risks.

Similarly, Deoria and Kushinagar, already known for their high rainfall, are becoming more vulnerable to seasonal flooding, drainage congestion, and associated crop damage. These districts represent zones of rainfall intensification, where adaptation will require robust flood-control infrastructure, improved drainage, and climate-resilient agricultural practices.

The central transitional belt, comprising Balrampur, Basti, Sant Kabir Nagar, and Siddharth Nagar, shows mixed patterns of both slight decline and marginal increases. For instance, parts of Balrampur register moderate declines (–124 mm to –81 mm), while Basti and Sant Kabir Nagar indicate smaller fluctuations close to stability (–40 mm to –3 mm). Siddharth Nagar displays minor increases in localized zones, highlighting its position as a transitional buffer between the arid west and flood-prone east. This belt remains particularly vulnerable as it faces both drought and flood risks, depending on seasonal variability. Integrated water management is therefore critical in this zone.

Interestingly, Maharajganj reflects a moderate to stable pattern, with changes ranging mostly between –40 mm and +40 mm. This relative stability compared to neighbouring districts suggeststhat rainfall variability here has been less pronounced, although its proximity to high-rainfall areas like Kushinagar and Deoria may still expose it to indirect flood impacts.

Hence, the spatial change detection for 2004–2024 underlines a dual climatic reality: While the west is becoming drier, the east and south are witnessing rainfall intensification. This divergence in rainfall dynamics poses contrasting challenges—aridity threatens agricultural productivity in the west, while excessive rainfall increases flood hazards in the east. The transitional districts in the centre face compounded risks and demand flexible strategies that address both extremes. The findings strongly call for district-specific adaptation measures: groundwater recharge and drought-proofing in the west, flood management in the east, and integrated resource management in the central belt. Without such targeted interventions, the changing rainfall regime could exacerbate vulnerabilities and undermine sustainable development in the Trans-Saryu region.

Conclusion and Recommendations

This study effectively examined the spatial heterogeneity and temporal variability of rainfall in the Trans-Saryu region with progressive aridity in the western districts and intensifying rainfall in the eastern districts, which requires district-specific policy interventions. In the West, priority should be given to strengthening irrigation infrastructure, adopting drought-tolerant cropping systems, and enhancing groundwater recharge mechanisms. Conversely, in the east, strategies must focus on flood control, drainage improvement, and the promotion of resilient agricultural practices. The central transitional belt, which faces exposure to both drought and flood risks, necessitates an integrated water management approach.

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Therapeutic potential of caffeic acid against alterations induced by combined exposure to Aluminum and Beryllium

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ABSTRACT

Metal contamination poses public health challenges around the world. This study evaluated therapeutic potential of caffeic acid against impairments induced by combined exposure to Aluminum and Beryllium in female albino rats. Rats were divided into five groups, group one served as control. Group two to five received combined dose of Aluminum nitrate (6.5 mg/kg, i.p.) and Beryllium nitrate (1 mg/kg, i.p.) daily for four weeks. Groups three to five received oral doses of caffeic acid at 10, 20 and 30 mg/kg, respectively for continuous two weeks of post- exposure.Behavioral performance was assessed 24 h after final administration of toxicants using elevated plus maze, T- maze and light and dark chamber. Rats exposed to toxicants showed pronounced anxiety-like behaviors and reduced time in dark compartment. Caffeic acid treatment significantly attenuated these effects in dose dependent manner, highlighted its neuroprotective roles against combined exposure to Aluminum and Berylliuminduced behavioral alterations.

Figure : 00 References : 30 Tables : 03
KEY WORDS : Aluminum, Beryllium, Caffeic acid, Neurotoxicity, Polyphenols.

Introduction

In the era of environmental pollution by toxic metals, much researches have focused on the toxicity of individual metals while real-world scenarios rarely involve exposure to a single metal. Living organisms are frequently exposed to mixtures of multiple metals simultaneously that create a complex interaction, which are not always predictable from single-metal exposure studies.Considerable studies have been conducted on heavy metals but comparatively less information is available on toxic effects after exposure to combination of light metals.

Aluminum is the third most abundant element in the Earth crust (~8%), widely used in electrical systems, leather processing, and water treatment and released naturally from soil, rocks and water under acidic conditions[20]. Aluminum induces oxidative stress, depletes antioxidant defense in vital organs,crosses blood-brain barrier, accumulates in neuronal tissues and disrupts calcium homeostasis[9]. It contributes neuroinflammation, protein aggregation and neurodegenerative disorders[15]. Beryllium is the lightest bivalent, low-densityhard metal with unique physical and chemical properties. Beryllium exposure generates reactive oxygen species leading to systemic toxicity[30].Beryllium exposure is linked to chronic lung disease (berylliosis), carcinogenesis, allergic, apoptosis, mutagenic action, neurotoxic effects and neuroinflammation disrupts blood brain barrier[23]. These effects are likely mediated by neuronal apoptosis, neurotransmitter imbalance and oxidative damage[25].

Caffeic acid(CA) is a plant derived hydroxy

TABLE-1: Therapeutic effect of caffeic acid on behavioral alterations

	Elevate Plus Maze			
Groups	**Total time spent in closed arm (Sec)**	**% time in closed arm**	**Total time spent in opened arm (Sec)**	**% time in opened arm**
Control	248.3± 13.7	82.76± 4.57	51.7± 2.85	17.23± 0.95
Al+Be	139.5± 7.71a	46.63± 2.57a	160.5± 8.87a	53.5± 2.95a
Al+Be+ CA 10	239.9± 13.2b	79.96± 4.42b	60± 3.31b	20± 1.10b
Al+Be+ CA 20	240.7± 13.3b	80.23± 4.43b	59.3± 3.27b	19.76± 1.09b
Al+Be+ CA 30	241.9± 13.4b	80.63± 4.45b	58.1± 3.21b	19.36± 1.07b
ANOVA	16.3^{Ω}	16.3^{Ω}	108^{Ω}	108^{Ω}

cinnamic acid that exhibits a diverse biological activity, including antioxidant, anti-inflammatory, antimicrobial, antiviral, anticancer, neuroprotective and metal chelating properties[17]. Its two phenolic hydroxyl (-OH) groups are responsible for its antioxidant activityto protect cells from oxidative damage. Carboxylic acid (-COOH) group contributes to slightly acidic medium that allows it to participate in chemical reactions involving proton exchange[29]. Caffeic acid improves memory learning and reduces anxiety and depression like behaviors by modulating neuro transmitters level, reducing neuro inflammation and preventing neuronal apoptosis[22].

Therefore, this investigation focused on the evaluation of therapeutic potential of caffeic acid against behavioral alterations induced by combined exposure to Aluminum and Beryllium.

Materials and Methods

Experimental animals and chemicals

Wistar female rats (160±10 g) were procured from All India Institute of Medical Science (AIIMS), New Delhi, India. Animals were maintained in polypropylene cages with provision of access to animal feed pellets and water *ad libitum*. The experimental study was approved by the Institutional Animal Ethics Committee (IAEC).All the animals were procured from recognized standard chemical dealers.

Experimental design

Thirty animals were randomly assigned into five groups having six animals in every group.

Group I: Control. **Group II:**$Al(NO_3)_3$ (6.5 mg/kg; i.p.) + $Be(NO_3)_2$(1 mg/kg; i.p.) for four weeks.**Group III:**$Al(NO_3)_3$ + $Be(NO_3)_2$(as in group II)+ CA 10 mg/kg (p.o., simultaneously for two weeks after two weeks exposure to toxicants). **Group IV:**$Al(NO_3)_3$ + $Be(NO_3)_2$ (as in group II)+ CA 20 mg/kg(p.o., simultaneously for two weeks after two weeks exposure to toxicants). **Group V:**$Al(NO_3)_3$ + $Be(NO_3)_2$ (as in group II)+ CA 30 mg/kg(p.o., simultaneously for two weeks after two weeks exposure to toxicants).

Behavioral studies

Elevated plus maze : The experimental device consisted of an elevated plus maze (40 cm above the floor) that consists of two opened and two enclosed arms, an open roof arranged in such a manner that two open arms were opposite to each other (four arms were 30 cm long and 5 cm wide). Rats were individually placed at the center of maze facinga closed arm. An entry into an arm was registered when animal placed all four paws on it. The parameters included number of entries and the percent time spent in closed and opened arms were recorded during a 300 second observation period. Data were expressed as the total time spent in closed arm (Sec), % time in closed arm and total time spent in opened arm (Sec), % time in opened arms[4].

T- maze : The T- maze was conducted over two phases. In phase one, animals were habituated to maize by allowing free exploration for 5 minutes with all arms accessible. In phase two, each animal underwent six trials; Trial phase: one goal arm was blocked, and the animal was placed in the start arm. Animal was allowed

TABLE-2 : Therapeutic effect of caffeic acid on behavioral alterations

	T- Maze	
Groups	**Correct alteration**	**% correct alteration**
Control	4± 0.22	66.6± 3.68
Al+Be	1.5± 0.08a	24.9± 1.37a
Al+Be+ CA 10	4± 0.22b	66.6± 3.68b
Al+Be+ CA 20	4± 0.22b	66.6± 3.68b
Al+Be+ CA 30	4± 0.22b	66.6± 3.68b
ANOVA	37.04^{Ω}	37.2^{Ω}

to explore opened goal arm and remained there for 20 seconds before being returned to its home cage. Choice latency: After a 30-second interval, both arms were opened, and the animal was placed again in the start arm. The animal's choice of arm was recorded, and entry into the arm opposite to the one visited during the trial phase was considered a correct alternation[8].

Light and dark chambers : Light and dark two distinct chambers, a dark chamber (20x 30x 35 cm) painted black and bright chamber painted white and brightly illuminated with white light sources. By observing the time duration of an animal which spent light-dark area in light and dark chamber, one can predict the anxiety or depression status of that animals[1].

Statistical analysis

The results were subjected to one way analysis of variance (ANOVA) followed by Tukey's *HSD post hoc* analysis at a significance level of 5%. All data were expressed as mean ± standard error. All statistical analyses were performed by using SPSS statistical software package(SPSS Inc, Chicago, IL).

Results

Elevated plus maze : Elevated plus maze test demonstrated significant behavior alteration among the group. Combined exposure to Aluminum and Beryllium group exhibited a drastic reduction in time spent in opened arm as well as % time in open were increased significantly as compared to control group as well as significantly decreased time spent and % time spent in closed arm. Different concentration of caffeic acid at 10, 20 and 30 mg/kg treatment reversed these effects in dose dependent manner. Group 10, 20 and 30 mg/kg showed significant restoration. *Tukey's* HSD *post hoc* test revealed that 30 mg/kg dose had maximum therapeutic efficacy (Table-1).

T-maze : T-maze exhibited significant decline in correct alteration behavior and percentage correct alteration indicating impaired spatial memory and cognitive functions in toxicity group. Caffeic acid treatment at 10, 20 and 30 mg/kg doses significantly restored performance towards control. Tukey's HSD *Post hoc* test revealed significant recovery at all three doses of caffeic acid for both correct alteration and % of correct alteration. These results suggested that caffeic acid markedly ameliorated aluminum and beryllium induced memory impairments (Table-2).

Light and dark chambers

Combined exposure to Aluminum and Beryllium reduced the time spent in the dark and correspondingly increased time spent in light suggesting heightened anxiety like behavior. Caffeic acid treatment led to a dose dependent improvement. Caffeic acid at 20 and 30 mg/kg dose showed significant improvement as compared to lower dose of caffeic acid 10 mg/kg. The ANOVA revealed significant differences in total time and percentage time spent in light, while time spent in dark and its percentage did not differ significantly. These findings suggested that caffeic acid effectively mitigated anxiety like behavior induced after combined exposure to Aluminum and Beryllium (Table-3).

Discussion

Metal toxicity is a major concern in occupational and environmental health problems. Aluminum and Beryllium are widely used as light metals; however, their

TABLE-3 : Therapeutic effect of caffeic acid on behavioral alterations

Light and Dark Chamber				
Groups	**Total time spent in dark (Sec)**	**% time in dark**	**Total time spent in light (Sec)**	**% time in Light**
Control	260± 14.4	86.66± 4.79	39.9± 2.20	13.3± 0.73
Al+Be	239± 13.2	79.93± 4.41	60.2± 3.32a	20.06± 1.10a
Al+Be+CA 10	240± 13.3	80.13± 4.42	59.6± 3.29a	19.86± 1.09a
Al+Be+CA 20	252± 13.9	84.26± 4.65	47.2± 2.60bc	15.7± 0.86bc
Al+Be+CA 30	256± 14.1	85.36± 471	43.9± 2.42bc	14.63± 0.80bc
ANOVA	0.54	0.53	13.0^{Ω}	13.0^{Ω}

exposure poses significant health risks. Neurotoxicity associated with these metals is well documented, involving oxidative stress, neuronal damage and cognitive impairment[26]. Both direct and indirect exposures to their salts impose adverse effect on the behavior and cognition, including attention, memory, learning capacity *etc*. Pathogenesis in neuro degeneration is linked to oxidative stress, which disrupts antioxidant defence and promotes neuronal cell death[3,20]. Plant derived molecules have ability to control reactive oxygen/nitrogen species, which may be utilized to treat memory impairments and cognitive problems. Caffeic acid has wide spectrum of pharmacological activities[17]. To mitigate toxic effect of Aluminum and Beryllium, caffeic acid was evaluated due to its strong antioxidant and metal chelating properties, which helped in reducing oxidative damage and metal induced toxicity[2].

Exposure to Aluminum nitrate and Beryllium nitrate resulted neurotoxicity in experimental animals as indicated by inability of rats to maintain equilibrium for 5 min in each trial on elevated plus maze[13]. Number of entries in opened and closed arms reflected the safety of closed arm. Reduction in % time spent, entry in opened arm and increased defections indicated high level of fear or anxiety. Anxiolytic xenobiotics enhances the number of entries and time spent in opened arms. Combination of toxicants decreased the number of entries and % time spent in closed arms showing anxiety effects. Improvement may be attributed to antioxidant of caffeic acid reduced neuronal damage and enhanced cognitive functions[9].

The T-maze is well established behavioral tool, used to asses spatial learning and memory in rats. Exposure to Aluminum and Beryllium impaired the spatial learning by increased latency and reduced alternative behavior[23]. This suggested neurotoxic effects, likely linked to oxidative stress. However, different doses of caffeic acid improved performance, indicating its neuroprotective role through antioxidant and metal chelating actions[5].

In light dark chamber, bright light acts as environment stress that minimizes the explorative behavior of rats. Reduction in the time spent, rearing behavior and number of entries in the light chamber was regarded as markers of anxiety[21]. Combined exposure to Aluminum and Beryllium decreased time spent, rearing behavior and number of entries in the light chamber, which confirmed anxiogenic effects after exposure to Aluminum and Beryllium[6].

On the basis of results, it may be concluded that all the three doses of caffeic acid *i.e.*, 10, 20 and 30 mg/kg recovered from behavioral alterations on a dose dependent manner and confirmed its therapeutic potential against behavioral alterations induced by combined exposure to Aluminum and Beryllium.

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Antifungal activity and polyphasic characterization of endophytic bacterial isolates obtained from Shamba *(Oroxylum indicum)*, an ethnomedicinal plant of Manipur, India

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ABSTRACT

A total of 57 isolates were obtained from root, stem and leaf samples of *Oroxylum indicum*. All the isolates were tested for antifungal activity against test fungal pathogens, *Rhizoctonia solani* (MTCC 4633) and *Pyricularia oryzea* (MTCC1477). Among 57, 8 (OInL1, OInL6, OInL11, OInL14, OInS13, OInS20, OInR13 and OInR15) and 11 isolates (OInL1, OInL6, OInL7, OInL11, OInL13, OInL14, OInS8, OInS20, OInR13, OInR17 and OInR1) exhibited antifungal activity against *Rhizoctonia solani* (MTCC 4633) and *Pyricularia oryzea* (MTCC1477) respectively. All isolates were also characterized for their polyphasic characteristics. Growth of the isolates was checked at different pH values (3, 4, 7, 9 and 10) and different salt (NaCl) concentrations (0, 2, 4, 6 and 8 % w/v). Optimal growth was observed at alkaline pH (7, 9 and 10) as growth was poor in acidic condition. Of 57 isolates, 56 grew well in all NaCl concentrations ranges tested except OInS19 which grew poorly with or without NaCl. Among 57 isolates, only one (OInS19)and 19 isolates (OInL1, OInL4, OInL6, OInL7, OInL11, OInL12, OInL13, OInL14, OInS2, OInS8, OInS20, OInR8, OInR12, OInR13, OInR14, OInR15, OInR16, OInR17 and OInR18) were found positive in MR and VP tests respectively. Furthermore, indole production, catalase activity, gelatin and starch hydrolysis test were performed for each bacterial isolate.

Figures : 04 References : 10 Table : 01

KEY WORDS : Antifungal activity, Endophytic bacteria, Oroxylum indicum, Polyphasic characterization

Abbreviation

MTCC: Microbial type culture collection

NA: Nutrient agar

SCNA: Starch Casein nitrate agar

MR and VP tests: Methyl red and Voges Proskauer test

PDA: Potato dextrose agar

Introduction

Endophytes are microorganism that inhabit internal plant tissue without causing harm to host plant.[5] They produce a plethora of bioactive compounds that have potential application in medicine, agriculture and industry.[10] The Global population doubled in the last 50 years and hence there is enormous demand of food supply to meet the requirement of the increasing population. The current approach being used involved utilizing chemical fertilizers and pesticides to increased yield; however it poses threats to environment.[3] So an alternative approach to combat these threats is to use microorganisms such as endophytic microbes as biocontrol agents.[2,9] over the past few decades, Biocontrol agents have become an alternative choice to control pests and disease affecting crop plants, which ensure sustainable agriculture along with environmental protection.[6] The biocontrol agents control diseases either

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TABLE-1 : Indole production test, catalase activity test, gelatin and starch hydrolysis test of bacterial isolates

S. No	Bacterial isolates	Methyl Red test	VP Test	Indole production test	Catalase activity	Gelatin hydrolysis test	Starch hydrolysis test
1	OInL1	_	+	+	+	+	+
2	OInL2	_	_	+	+	+	_
3	OInL3	_	_	+	+	+	_
4	OInL4	_	+	-_	+	+	_
5	OInL5	_	_	+	+	+	_
6	OInL6	_	+	+	+	+	+
7	OInL7	_	+	+	+	+	+
8	OInL8	_	_	+	+	+	_
9	OInL9	_	_	+	+	+	_
10	OInL10	_	_	+	+	+	_
11	OInL11	_	+	+	+	+	+
12	OInL12	_	+	+	+	+	+
13	OInL13	_	+	+	+	+	_
14	OInL14	_	+	+	+	+	+
15	OInL15	_	_	+	+	+	_
16	OInS1	_	_	+	+	+	_
17	OInS2	_	+	+	+	+	+
18	OInS3	_	_	+	+	+	_
19	OInS4	_	_	+	+	+	_
20	OInS5	_	_	+	+	+	_
21	OInS6	_	_	+	+	+	_

S. No	Bacterial isolates	Methyl Red test	VP Test	Indole production test	Catalase activity	Gelatin hydrolysis test	Starch hydrolysis test
22	OInS7	_	_	+	+	+	_
23	OInS8	_	+	+	+	+	_
24	OInS9	_	_	+	+	+	_
25	OInS10	_	_	+	+	+	_
26	OInS11	_	_	+	+	+	_
27	OInS12	_	_	+	+	+	_
28	OInS13	_	_	+	+	+	_
29	OInS14	_	_	+	+	+	_
30	OInS15	_	_	+	_	+	+
31	OInS16	_	_	+	+	+	_
32	OInS17	_	_	+	+	+	_
33	OInS18	_	_	+	+	+	_
34	OInS19	+	_	+	_	+	_
35	OInS20	_	+	+	+	_	_
36	OInR1	_	_	+	+	+	_
37	OInR2	_	_	+	+	+	_
38	OInR3	_	_	+	+	+	_
39	OInR4	_	_	+	+	+	_
40	OInR5	_	_	+	+	+	_
41	OInR6	_	_	+	+	+	_
42	OInR7	_	_	+	+	+	_
43	OInR8	_	+	+	+	+	_
44	OInR9	_	_	+	+	+	_

S. NO	Bacterial isolates	Methyl Red test	VP Test	Indole production test	Catalase activity	Gelatin hydrolysis test	Starch hydrolysis test
45	OInR10	_	_	+	+	+	_
46	OInR11	_	_	+	+	+	_
47	OInR12	_	+	+	+	+	_
48	OInR13	_	+	+	_	+	+
49	OInR14	_	+	+	+	+	_
50	OInR15	_	+	_	+	+	+
51	OInR16	_	+	+	+	_	_
52	OInR17	_	+	+	_	+	_
53	OInR18	_	+	+	+	+	_
54	OInR19	_	_	+	+	+	_
55	OInR20	_	_	+	+	+	_
56	OInR21	_	_	+	+	+	_
57	OInR22	_	_	+	+	+	_

by direct/indirect interaction with causal agents. The biocontrol agents control diseases by direct interaction with causal agents through nutrient competition or parasitism or by indirect interaction with causal agent through stimulation of plant defense mechanisms against various pests and diseases.[1]

Oroxylum indicum is an endangered rare medicinal plant used in the treatments of many ailments in ayurvedic, herbal and folk medicines. Each plant part possessed medicinal values such as antimicrobial, antifungal, antioxidant, anti-inflammatory, anticancer *etc.*[8] In this study, endophytic bacterial isolates were isolated from leaf, stem and root samples of *Oroxylum indicum.* All 57 isolates were screened for antifungal activity against test fungal pathogens, *Rhizoctonia solani* and *Pyricularia oryzea* using *in vitro* Dual culture method. All 57 isolates were also checked for various polyphasic characteristic like Indole production, catalase activity, gelatin and starch hydrolysis test. Furthermore, growth of all bacterial isolates at different salt concentrations and pH ranges were checked.

Material and Methods

1. Isolation of endophytic bacteria : The leaf, stem and root samples of the ethnomedicinal plant of Manipur, *Oroxylum indicum* was collected in November 2018 from Khurkhul, Imphal west, Manipur (24.93°N, 93.87°E). The samples were washed thoroughly under running tap water and surface sterilized by sequential treatment with the following solutions: 4-10 min wash in 5% sodium hypochlorite, 10 min wash in 2.5% $Na_2S_2O_3$, 5 min wash in 75% Ethyl alcohol followed by washing in distilled water with a final rinse in 10% $NaHCO_3$.[7] Isolation was done on different media viz. Nutrient Agar (NA), Starch Casein Nitrate Agar (SCNA).

2. Test fungal pathogens: Test fungal pathogens *viz. Rhizoctonia solani* (MTCC 4633) and *Pyricularia oryzea* (MTCC 1477) were procured from Microbial type Culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The strains were grown and maintained on Potato dextrose agar (PDA plate).

3. Biocontrol assay against fungal pathogen (Dual Culture Method) : All the isolates were checked for antifungal activity against *Rhizoctonia solani* (MTCC 4633) and *Pyricularia oryzea* (MTCC 1477) by **Dual Culture Method**.[4] This test was done on PDA (Potato Dextrose Agar) plates. Agar wells (6 mm diameter) were made on PDA plates using sterilized cork borer and then, agar plugs from the freshly grown bacterial isolates were placed on punched wells in duplicate and kept at 30°C for 48 hr. Also, agar plug of the test fungal pathogen was placed at the centres of labelled plates. A plate containing test fungal agar plug without any isolate plug was kept as controls. All test plates were kept at 30°C until the control plate show full growth.

The Percentage growth inhibition was calculated using the following formula:

$$\text{Growth inhibition} = \frac{R1 - R2}{R1} \times 100$$

Where, R1=Radius of fungal growth in control plate;
R2= Radius of bacterial growth in test plate

4. Biochemical characterization :

4.1 Methyl red (MR) and Voges Proskauer (VP) Tests: MR-VP medium is used for differentiating coliform bacteria particularly coli-aerogenes group. During dextrose fermentation, some bacteria cause high acidity of the medium while others produce low acidity. The test to detect high acidity end product is known as the MR test while the other test to detect low acidity product (formation of acetyl methyl carbinol *i.e.* acetoin) is called the VP test.

Dextrose is a fermentable carbohydrate. Some bacteria convert glucose to pyruvate by the EMP pathway. Some bacteria produce large quantity of acid such as lactic acid, acetic acid and formic acid by metabolizing pyruvate using mixed acid pathway that lowers the pH of the medium to below 4.4 while other species produce less acid so the fall in pH is less. This difference can be seen using methyl red, which appears yellow above pH 5.1 and red at pH 4.4. Other bacteria metabolize pyruvate through butylene glycol pathway and produce acetoin like neutral end product (pH>6). Acetoin is oxidised in presence of oxygen and potassium hydroxide to diacetyl, and react with creatine to give red colour. This test to detect less acid end product was performed as per the procedure.

Procedure: A loopful of bacterial isolates were inoculated in 10 ml MR-VP medium in two tubes and incubated for 2-3 days at 30!.

For MR Test, 2-3 drops of methyl red indicator solution was added to each tube.

For VP test, 600µl each of Baritt¼s reagents A and B was added to each tube.

Red colouration confirms positive result for MR-Test while pink to red colouration indicates positive result for VP test.

4.2 Indole test : All isolates were inoculated in 5ml of Nutrient agar media and kept incubated for 48 hrs at 30!. Then, we checked Indole production from tryptophan with the help of the enzyme tryptophanase by adding 600 ml of Kovac's reagent.

4.3 Catalase activity : Catalase is an enzyme which is produced by microorganisms that live in oxygenated environments to neutralize H_2O_2. Catalase activity of all isolates was checked by mixing small inoculums of bacterial isolate with 3% v/v H_2O_2. Formation of oxygen bubbles confirms the positive result.

4.4 Hydrolysis of Gelatin : Gelatin iron agar medium

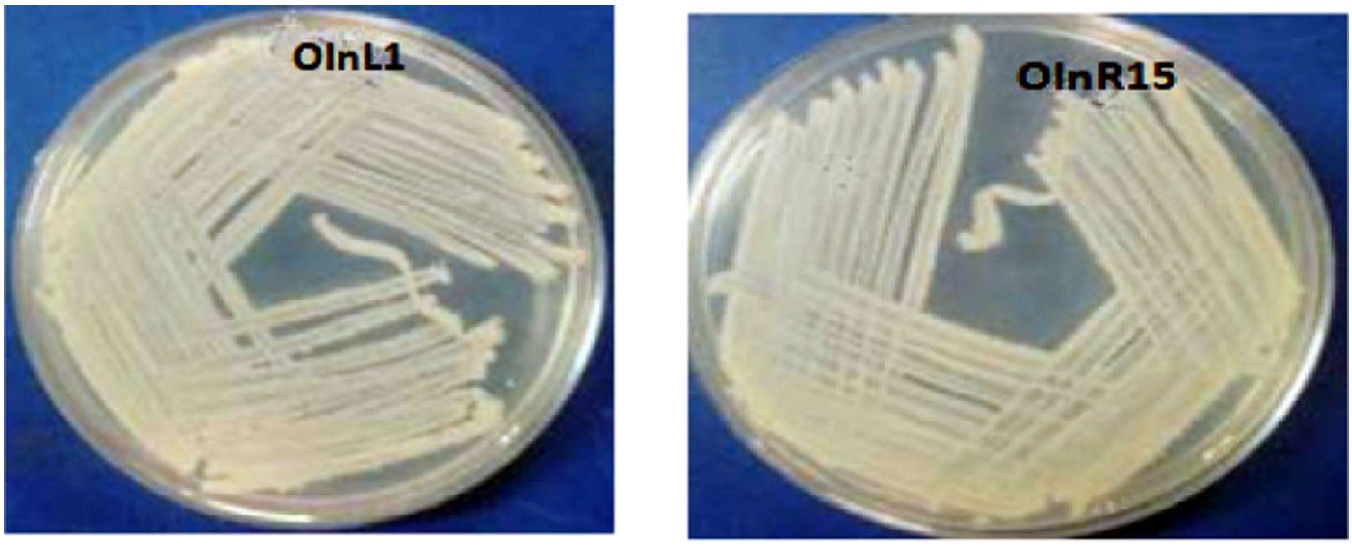

Fig. 1 : Representative Plates showing pure cultures of bacterial isolates from *Oroxylum indicum*

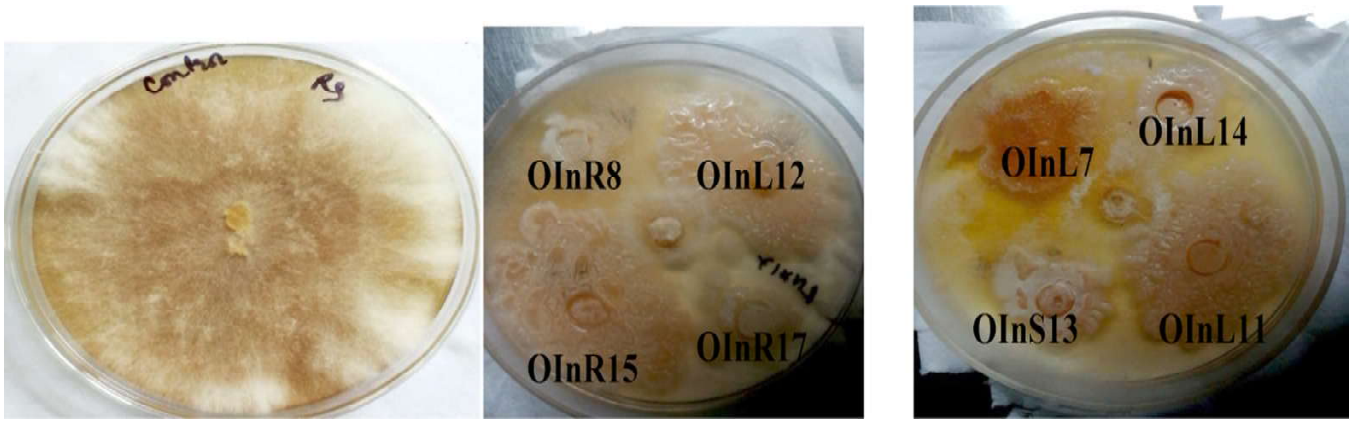

Fig. 2 : Antagonistic activity against *Rhizoctonia solani* (MTCC 4633)

was inoculated with a loopful of actively growing isolates and allowed to grow for 6–7 days at 30!. Control tubes solidified when kept in ice whereas inoculated tubes remained unsolidified, which confirm hydrolysis of gelatin.

4.5 Starch hydrolysis test : NA plates supplemented with 0.3 % soluble starch were inoculated with actively growing isolates and allowed to grow at 30! for 3 days. A clear yellow zone formed around the inoculation spots indicates hydrolysis of starch when flooded with Gram's iodine.

5. Physiological Characterization:

5.1 Effect of salt on bacterial growth

Procedure:

- NA media with different salt concentrations were prepared (0, 2, 4, 6 and 8% w/v NaCl)
- With a loop, 4 different cultures were inoculated in the 4 sectors of the divided plate.
- Incubated aerobically at 30! and growth was observed after 3-4 days of incubation.
- Growth indicates positive reaction.

5.2 Effect of pH on growth

Procedures:

- NA media of varying pH (3, 4, 7, 9 and 10) were prepared.
- With a loop, the cultures were inoculated in the 4 sectors of the media plates.
- Incubated aerobically at 30! and growth was observed after 3-4 days of incubation.
- Growth indicates positive reaction.

Result and Discussion

1. Isolation of bacterial isolates

Healthy leaf, stem and root parts of the medicinal plant, *Oroxylum indicum,* were used to isolate endophytic bacteria isolates using different isolation media *viz.* NA and SCNA. A total of 57 endophytic bacterial isolates were collected. 15 isolates were obtained from leaf samples, 20 from stem samples and 22 from root samples. Fig.1 Shows two representative endophytic bacterial isolates (OInL1 and OInR15) obtained from leaf and root sample of *Oroxylum indicum.*

Each bacterial isolate was given a unique Code

Fig. 3: Antifungal activity against *Pyricularia oryzea* (MTCC1477)

Fig. 4: Starch hydrolysis test of some bacterial isolates

for identification and maintained in the laboratory. 15 isolates were obtained from leaf samples labelled as OInL1-OInL15, 20 from stem samples (OInS1-OInS20) and 22(OInR1-OInR22) from root samples using two different media, NA and SCNA.

2. Antifungal activity against test fungal pathogens

All 57 bacterial isolates were screened for antifungal activity against test fungal pathogen *Rhizoctonia solani* (MTCC 4633) and *Pyricularia oryzea* (MTCC1477). Eight isolates (OInL1, OInL6, OInL11, OInL14, OInS13, OInS20, OInR13 and OInR15) showed antifungal activity against *Rhizoctonia solani* (MTCC 4633) (Fig. 2). Highest activity was shown by OInR15 with 82% relative growth inhibition followed by OInL6 and OInL12 with 67%. Moreover, 11 isolate (OInL1, OInL6, OInL7, OInL11, OInL13, OInL14, OInS8, OInS20, OInR13, OInR17 and OInR18) showed antifungal activity against Pyricularia oryzea (MTCC 1477) (Fig. 3).OInS8 showed highest growth inhibition with 83% followed by OInL7, OInL11, OInS20, and OInR18 with 68%.

3. Biochemical characterization

All 57 endophytic isolates from *Oroxylum indicum* were further characterized biochemically using MR and VP Tests. Only one isolate, OInS19 was found positive in MR while 19 isolates (OInL1, OInL4, OInL6, OInL7, OInL11, OInL12, OInL13, OInL14, OInS2, OInS8, OInS20, OInR8, OInR12, OInR13, OInR14, OInR15, OInR16, OInL17 and OInR18,) were positive in VP Tests (Table-1).

All 57 isolates were also tested for Indole production, catalase activity, gelatin and starch hydrolysis (Table-1). Indole production was checked by addition of Kovac's reagent which contain aldehyde group. Deep golden red ring indicates a positive reaction. 55 isolates (except OInL4 and OInR15) out of 57 were positive for Indole production. Catalase activity of all isolates was checked by adding a drop of 3 % H_20_2 over the bacterial colony. Except four(OInS15, OInS19, OInR13 and OInR17), all isolate showed Catalase activity. Furthermore, 55 isolates were positive for gelatin hydrolysis. In starch hydrolysis test, 10 isolates (OInL1, OInL6, OInL7, OInL11, OInL12, OInL14, OInS2, OInS15, OInR13 and OInR15,) were positive in starch hydrolysis test. Clear zone formation around bacterial colonies on flooding with iodine solution indicates starch hydrolysis (Fig. 4).

4.1. Growth of bacteria at different salt concentration

56 isolates could grow at all tested NaCl concentrations (0-8%). Except, one isolate (OInS19) showed poor growth in the salt concentrations used in the assay as the isolate might require special media for growth.

4.2 Growth of bacteria at varying pH ranges

All 57 isolates were also tested for pH tolerance. All isolates showed optimal growth at pH 7-10, however couldn't grow in acidic pH (pH 3 &pH 4).

Conclusion

Among 57, 8 bacterial isolates (OInL1, OInL6, OInL11, OInL14, OInS13, OInS20, OInR13 and OInR15) Showed antifungal activity against *Rhizoctonia solani* (MTCC 4633) whereas 11 isolate (OInL1, OInL6, OInL7, OInL11, OInL13, OInL14, OInS8, OInS20, OInR13, OInR17 and OInR18) showed activity against *Pyricularia oryzea* (MTCC1477). Out of 57 isolates, 55isolates were found positive for Indole production. Except four, all isolates showed Catalase activity. Furthermore, 55 and 10 isolates (OInL1, OInL6, OInL7, OInL11, OInL12, OInL14, OInS2, OInS15, OInR13 and OInR15) were positive in gelatin and starch hydrolysis test respectively.

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Hematological variables and electrolytes experience, toxicological changes on combined exposure to Aluminum and Beryllium

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ABSTRACT

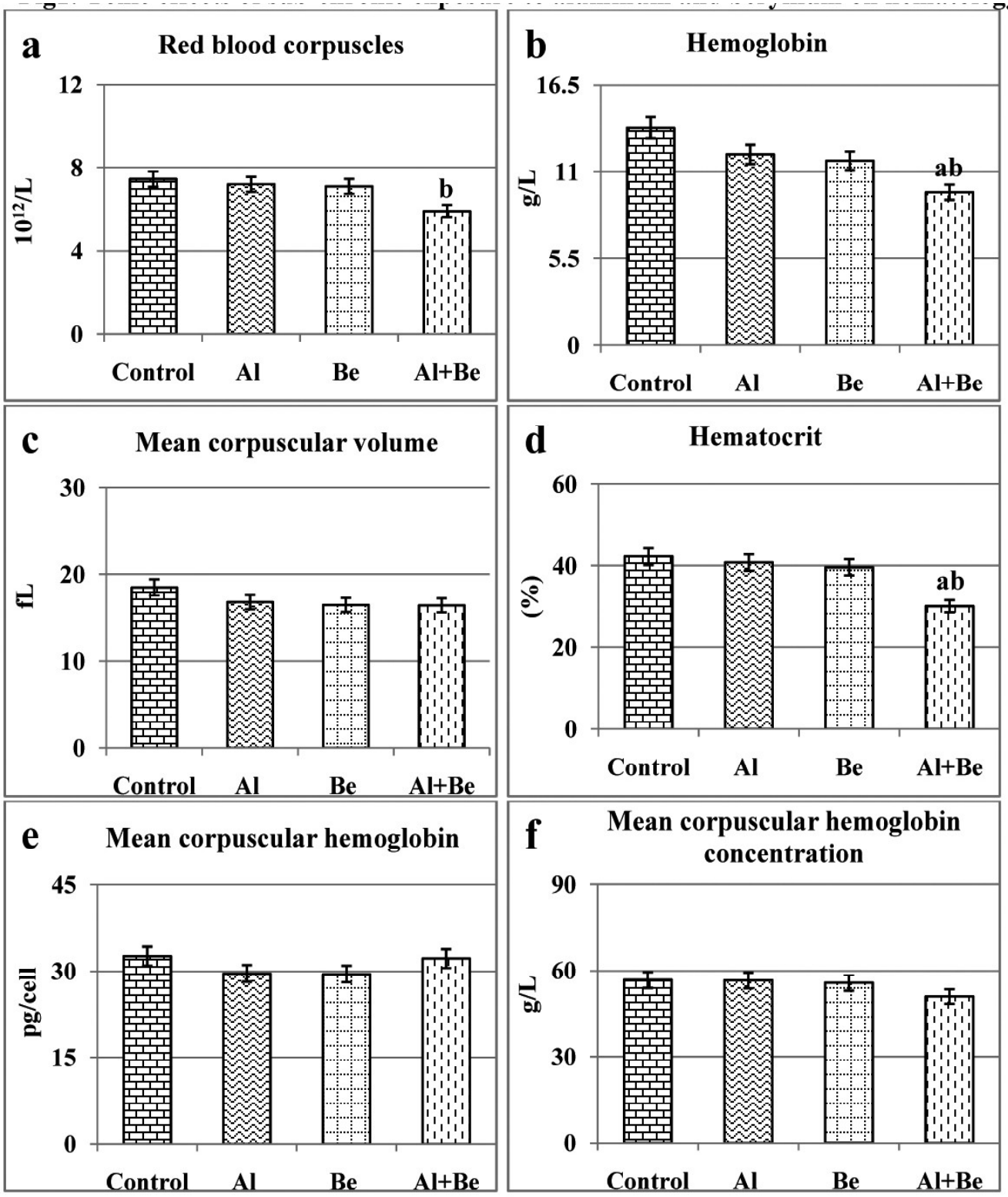
Aluminum (Al) and Beryllium (Be) are light metals, widely used across various sectors. The present study was conducted to investigate toxic effects of Al and Be alone and their combination on hematology and electrolytes in female albino rats. Rats were administered with aluminum nitrate 6.5 mg/kg, *i.p.* and beryllium nitrate 1 mg/kg, *i.p.* for continuous 28 days followed by rest for 07 days. On 36th day, animals were euthanized; blood was collected through retro-orbital venous sinus for hematology and electrolytes. The findings revealed alterations in RBCs, WBCs, Hb, PLT, MCV, HCT, PLCR, MCH, MCHC, PCT and MPV. Significant variations in Na^+, K^+, Cl^- and iCa^{2+} ions were noted in electrolytes in serum. Thus, it can be concluded that individual and combined exposure to Al and Be exert toxic effects by remarkable alterations in hematology and electrolytes; however, combined exposure imposes more pronounced toxic effects.

Figures : 03 References : 34 Table : 00
KEY WORDS : Aluminum, Beryllium, Electrolyte, Hematology

Introduction

Metal pollution is the introduction of toxic elements into the environment through natural and anthropogenic activities that lead adverse effects on living organisms[12]. This poses health problems to humans by disrupting normal physiological functions. Occupational sources of metal exposure include coal mining activities, metallurgy, nuclear power plants, pesticides, smelting and fertilizers industries, which impose significant health risks to the workers and population surviving near these industrial areas[13]. At one side, Pb, Hg, Cd, and As *etc.*, are among the most commonly released heavy metals due to anthropogenic activities and induce severe toxicity among humans. These metals enter the body *via* inhalation, ingestion and dermal contact and generate reactive oxygen species (ROS), suppress antioxidant enzymatic functions, disturb protein functioning, impair enzymatic activities, and alter DNA molecules and their effects[16]. On the other hand, Aluminum (Al) and Beryllium (Be) are light metals, widely used in aerospace, spacecrafts, electronics, nuclear industry and metallurgy from where these are released into the environment[29,30]. Aluminum generally accumulates in vital organs, inhibits enzymatic activities, disturb cellular metabolism by passing through blood-brain barrier[2], leads to neurotoxicity, hemo-toxicity and immune dysfunctions[22]. Beryllium produces toxicity, particularly by affecting lungs to cause chronic beryllium disease (CBD)[14], strongly immuno-toxic[5], alters blood profile[5], suppresses antioxidant enzymes and accumulates in liver, kidney and brain to cause organ toxicity[18,19,20].

Use of Al and Be in various technological purposes and combustion of coal during many industrial activities poses risk of exposure to Al and Be to human and animals both. Thus, it seems essential to study toxic consequences after combined exposure to Al and Be



Data are presented as mean ± SE (n=6); [a]Control vs Al, Be, Al+Be at P≤ 0.01; [b]Control vs Al, Be, Al+Be at P≤ 0.05; [@]significant for ANOVA

Parameters	RBC	Hb	MCV	HCT	MCH	MCHC
F Variance	3.836[@]	7.844[@]	1.308	8.049[@]	1.117	0.983

Fig. 1 : Toxic effects of sub-chronic exposure to aluminum and beryllium on hematology

and establish a multisystem toxicity model for combination of Al and Be. Workers are prone to be co-exposed to both Al and Be, making the study of their combined toxicity environmentally and occupationally relevant.

Thus, this investigation aimed to evaluate sub-chronic toxicity, induced after individual and co-exposure to Al and Be in rats considering hematology and serum electrolytes.

Materials and Methods

Ethical approval and animal maintenance

Animal experiments were conducted in accordance with the guidelines of CPCSEA and experimental design was approved by the institutional animal ethics committee (CPCSEA/GO/Re/S/06). Healthy female Wistar rats (10-12 weeks old having 160±10g body weight) were housed under standard husbandry conditions in cleaned and disinfected polypropylene cages. They were provided standard pelleted rat feed obtained from Akhoorath Ventures Pvt. Ltd., Dehradun, Uttarakhand, India and free access to drinking water.

Experimental design

Aluminum nitrate [$Al(NO_3)_3$] was dissolved in distilled water making up doses of 6.5 mg/5 ml/kg and administered through *i.p.* route[1]. Beryllium nitrate [$Be(NO_3)_2$] was dissolved in distilled water making up doses of 1 mg/5 ml/kg and administered intraperitoneally (i.p.)[33]. Experimental design was as following:

Group 1: Received vehicle 5 ml/ kg, *i.p.* for continuous 28 days and considered as control.

Group 2: Received $Al(NO_3)_3$ 6.5 mg/kg, *i.p.* for continuous 28 days.

Group 3: Received $Be(NO_3)_2$ 1 mg/kg, *i.p.* for continuous 28 days.

Group 4: Received combination of $Al(NO_3)_3$ (6.5mg/kg, *i.p.*) and $Be(NO_3)_2$ (1mg/kg, *i.p.*) for continuous 28 days.

Animals from all the groups were administered vehicle orally for next 07 days. On 36th day, animals were subjected to mild ether anesthesia. Blood samples from all the animals were collected in EDTA coated tubes for hematology and in another set of regular glass vials to isolate serum for electrolyte analysis.

Hematology and electrolyte analysis

Hematology included RBCs, WBCs, platelet (PLT), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet large cell ratio (PLCR), platelet large size ratio (PLSR), mean platelet volume (MPV) and procalcitonin (PCT) was carried out using biogeny fully automatic bonavera count hematology analyzer. Serum electrolytes, including Sodium ions (Na^+), potassium ions (K^+), chloride ions (Cl^-), and ionized calcium (Ca^{2+}) were analyzed using sens-e-lyte ARK diagnosis ISE electrolyte fully automatic analyzer as per manufacturer's instructions.

Statistical analysis

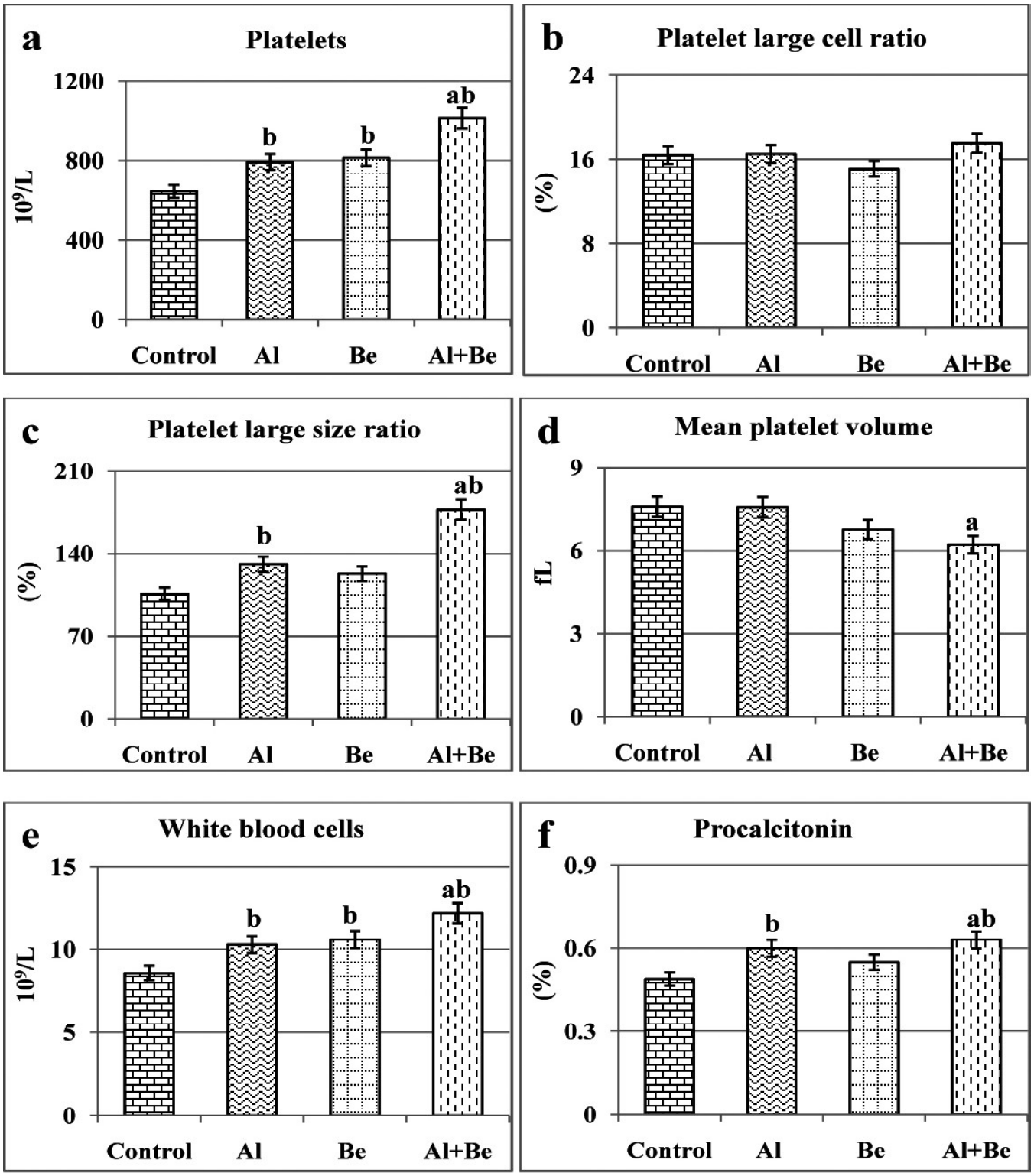
Results were expressed as mean ± SE (n = 6) and statistically analyzed through one-way analysis of variance (ANOVA) followed by student's t-test to determine the significant differences between two groups at $P \leq 0.01$ and $P \leq 0.05$[31].

Results and Discussion

Hematology

Fig. 1 (a-f) illustrates toxic consequences after exposure to Al, Be and its combination on RBCs, Hb, MCV, HCT, MCH and MCHC. Combined exposure to Al and Be elicited significant decrease in RBCs, Hb and HCT as compared to individual exposure to Al and Be ($P \leq 0.01$; $P \leq 0.05$). These effects could be due to Al induced ROS, which initiated peroxidation of RBC membrane lipids, resulting in increased membrane fragility and hemolysis[28]. Exposure to Al disturbed iron metabolism and activity of erythropoietin, thereby declined the production of RBCs in bone marrow[7,8]. The Al^{3+} competes with Fe^{3+} at transferrin binding sites, decreasing its availability for hemoglobin synthesis and ultimately lowering HCT levels[24]. On the other hand, Be^{2+} forms complexes with proteins, triggered autoimmune hemolysis, and suppressed bone marrow formation leading to declination in RBCs, hemoglobin and hematocrit levels[21]. Thus, dual interference of Al and Be in iron metabolism and heme synthesis enzymes markedly reduced RBCs, hemoglobin, and hematocrit. No statistical difference was found in MCV, MCH and MCHC levels.

Fig. 2 (a-f) demonstrates levels of PLT, PLCR, PLSR, MPV, WBCs, and PCT. The PLT and WBCs showed significantly increased level after exposure to Al and Be individually as well as in combination of Al and Be when compared to control at $P \leq 0.01$ and $P \leq 0.05$. The PLSR and PCT revealed significantly increased level in Al and combination of Al and Be group at $P \leq 0.05$, whereas combination of Al and Be showed significant difference at $P \leq 0.01$. Significant fall was noticed in MPV in combination of Al and Be group at $P \leq 0.01$. No significant alteration was noticed in any group in case of PLCR. Accumulation of Al could suppress



Data are presented as mean ± SE (n=6); [a]Control vs Al, Be, Al+Be at P≤0.01; [b]Control vs Al, Be, Al+Be at P ≤ 0.05; [@]significant for ANOVA

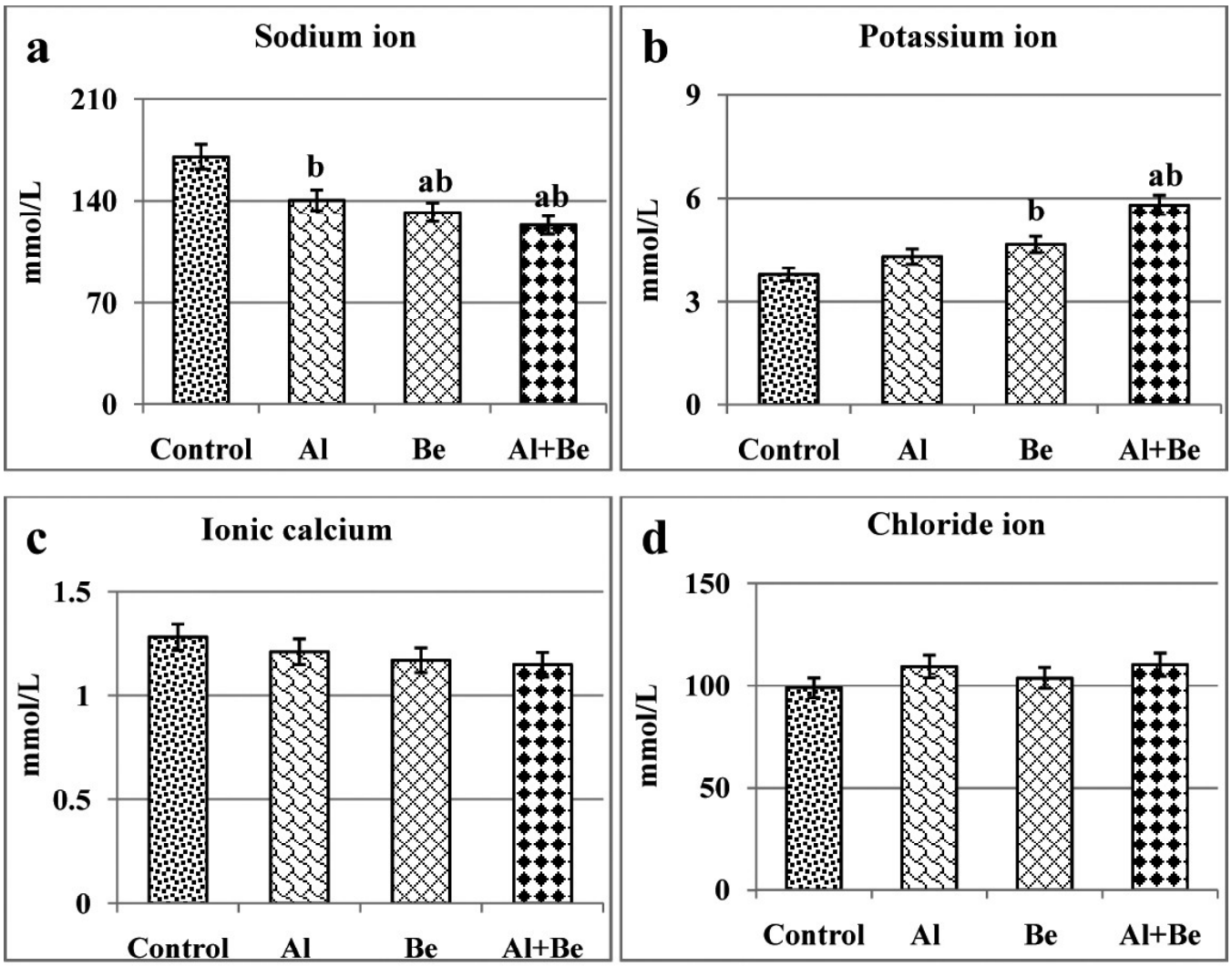
Parameters	PLT	PLCR	PLSR	MPV	WBCs	PCT
F variance	19.464[@]	1.415	19.464[@]	3.470[@]	7.745[@]	4.543[@]

Fig. 2 : Toxic effects of sub-chronic exposure to aluminum and beryllium on hematology and procalcitonin.

megakaryocyte function, resulting in decreased production and maturation of platelets[6,4]. Oxidative damage due to combination of Al and Be could induce fragmentation of large platelets that decreased MPV levels[15]. Decreased MPV level reflects an immune-mediated inflammatory response[27]. The combination of Al and Be could enhance oxidative stress[26,34], thus, excessive lipid peroxidation in RBCs and bone marrow remarkably increased oxidative stress, which stimulated bone marrow to produce more WBCs[10,23]. The Al and Be bind to proteins and are recognized as antigens, which further stimulates immune response. Increased platelet counts suggested an elevated rate of megakaryopoiesis and thrombopoietin activity. Under toxic conditions, large, immature, and hyperactive platelets are released from the bone marrow, indicating platelet activation during inflammatory responses, which collectively raised PLSR[3]. Bacterial infections, tissue damage and sepsis are typical conditions associated with elevated procalcitonin levels[11,25].

Serum electrolyte analysis

Electrolytes play a vital role in nerve signaling, muscular coordination, enzymatic activity, fluid, pH balance and cellular homeostasis[17]. Fig. 3 indicates toxic consequences on serum electrolytes after exposure to



Data are presented as mean ± SE (n=6); [a]Control vs Al, Be, Al+Be at P≤ 0.01; [b]Control vs Al, Be, Al+Be at P≤ 0.05; [@]significant for ANOVA				
Parameters	Na^+	K^-	Ca^{2+}	Cl^-
F variance	8.06[@]	10.08[@]	0.89	0.977

Fig. 3: Status of serum electrolytes after aluminum and beryllium induced toxicity

Al^{3+}, Be^{2+} and their combination. Exposure to Al alone significantly decreased Na^+ level in serum at $P \leq 0.05$, whereas Be and combination of Al and Be significantly decreased Na^+ level in serum both at $P \leq 0.01$ and $P \leq 0.05$ (fi. 3 a). Reduced Na^+ level in serum was due to disrupted cell membranes and interference of Al and Be in sodium-potassium pump, which allowed more Na^+ ions to leak into the cells, decreasing its level in the blood[9]. Significantly elevated K^+ level was observed after exposure to Be alone at $P \leq 0.05$, whereas combination of Al and Be both at $P \leq 0.01$ and $P \leq 0.05$) (fig. 3 b). Exposure to Al and Be could lead to metabolic acidosis, where the blood becomes highly acidic. In response to acidosis, K^+ ions shift from inside cells into the bloodstream, raising K^+ in serum[32]. No significant alteration was noticed Ca^{2+} and Cl^- level in serum (fig. 3 c-d).

Conclusion

Sub-chronic exposure to Al and Be and their combination induced significant alterations in some of the hematological parameters and serum electrolyte levels that reflected early sign of systematic toxicity. Thus, exposure to combination of Al and Be exerted more toxic effects as compared to their individual exposure even at low doses.

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