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Contents

RESEARCH ARTICLE

COPPER AND CADMIUM SULPHIDE NANOPARTICLES CAN INDUCE MACROMUTATION IN *NIGELLA SATIVA* L. (BLACK CUMIN)

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Abstract: Dry seeds(moisture content: 5.0%) of *Nigella sativa* L. (Family: Ranunculaceae; common name- black cumin, spice of commerce with immense therapeutic uses) are exposed to chemically synthesized copper (Cu) and cadmium sulphide (CdS) nanoparticles (NPs) at the doses of 0.25, 0.50 and 1.00 µg/ml for 3 and 6 h durations . EMS (ethyl methanesulphonate–0.25, 0.50 and 1.00%, 3 and 6 h durations) and gamma irradiations (25, 50, 100, 200 and 300 Gy; ^{60}Co source) are used as positive control. The objective of the work is to foresee whether NPs can induce stable phenotypic mutation. The present communication highlights macromutation types and frequency, mutagenic efficiency and effectiveness and meiotic chromosome behaviour in treated materials and suggests the efficacy if NPs in inducing mutation in *N.sativa* and crop improvement.

Keywords: Cu- and CdS-NPs, Macromutants, Meiotic analysis, Mutagenic efficiency and effectiveness, *Nigella sativa*

INTRODUCTION

The nanoparticles (NPs) are characterized by their small size and large surface and large surface area (ranging between 1 and 100 nm–Roco 2003) and possess unique physico-chemical properties (Remédios*et al.* 2012; Masarovičová and Králová 2013). NPs has global significance due to its wide application in industry, medicine, biotechnology, agriculture, different aspect of life sciences among others (Roco 2003, Lam *et al.* 2004, Caruthers *et al.* 2007, Nowack and Bucheli 2007, Scrinis and Lyons 2007, Singh *et al.* 2008, Nair *et al.* 2010, Castiglione *et al.* 2011,Remédios*et al.* 2012). Most significantly, Halder*et al.* (2015 a, b) reported that chemically synthesized copper (Cu) and cadmium sulphide (CdS) NPs can induce stable heritable changes (macromutation) in *Macrotylomauniflorum* (Lam.) Verdc (Family: Leguminosae). Furthermore, Kumbhakar*et al.* (2016) highlighted the potentiality of Cu- and CdS-NPs as mutagenic agents in *Nigella sativa* L. (Family: Ranunculaceae; common name- black cumin) as a test material. Such findings trigger the essentiality to attain further scientific knowledge from plant system on induced mutagenesis following NPs treatment. With the view to it, the present investigation has been designed and describes the mutagenic efficiency, mutagenic effectiveness and macromutation types, frequencies and their meiotic chromosome behaviour in *N. sativa*L. (spice yielding plant of commerce with immense therapeutic uses-Datta*et al.* 2012) following treatments with chemically synthesized copper and cadmium sulphide nanoparticles in comparison with the conventional mutagens namely, ethyl methanesulphonate (EMS) and gamma irradiation. The objective of the present study is to assess whether NPs can induce similar genetic variations as that of the well established mutagens under study.

MATERIAL AND METHOD

Germplasm

Seed samples of *Nigella sativa* L. (Ranunculaceae) were collected from Medicinal Plant Garden, NarendrapurRamkrishna Mission, Government of West Bengal, India. The moisture content of the mother seed stock was determined as 5.0%.

Synthesis and characterization of NPs

Cu- and CdS-NPs were prepared using wet chemical co-precipitation methods as described earlier by Chatterjee *et al.* (2012) and Halder*et al.* (2015 b) respectively.The NPs were characterized using UV–visible spectra (UV-vis), Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD), Dynamic Light Scattering (DLS), Field emission scanning electron microscopy (FESEM) and Transmission electron microscopy (TEM)for assessment of their nature and size (Kumbhakar*et al.* 2016). Opto-physical studies of the prepared NPs confirm nano standard quality (Kumbhakar*et al.* 2016).

Treatments

Dry and filled seeds of *N. sativa* were exposed to the prepared solutions of Cu- and CdS-NPs (0.25, 0.50 and 1.00 µg/ml, 3 and 6 h durations) and EMS (0.25, 0.50 and 1.00 %, 3 and 6 h durations; solution prepared in 0.2 M phosphate buffer, pH 6.8). Dry seeds were also exposed to gamma irradiation doses (25, 50, 100, 200 and 300 Gy, source ^{60}Co,

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absorbance dose rate 47.4 Gy/min, source to distance 12 cm). Dry control and bulk Cu- and CdS controls (0.25 µg/ml, 3 h) were also kept for assessment. Each lot of treatment comprised of 100 seeds.

Assessment of biological damages

Biological damages (M_1 generation attributes) like lethality, injury and sterility were ascertained from seed germination, seedling growth and seed yield per plant respectively. The extent of lethality and injury were determined from the relative reduction of seed germination frequency and seedling growth in treated samples (under controlled Petri plate conditions- as was suggested by Konzak*et al*. 1965) as compared to controls (per cent of control). Seed sterility was assessed in each treatment (seeds of each plant was weighed on harvest) and is represented as percentage of reduction in seed weight in treatment in relation to controls (Dubey and Datta 2014).

Raising of M_1 and M_2 plant population

Fifty seeds from each treatment including controls were sown in the experimental field plots of Department of Botany, University of Kalyani (West Bengal plains, Nadia, latitude 22˚50′ to 24˚11′ N, longitude 88˚09′ E to 88˚48′ E, elevation 48 ft above sea level, sandy loamy soil, pH 6.85) in late November to raise M_1 plant population. The plants were harvested in mid-April.

Selfed seeds (first formed flower was bagged in each case) of each surviving M_1 plants were harvested separately and were used to raise M_2 (plant to row) generation. Control lines were also grown. Plants were grown having 15 cm between plants and 25 cm between rows. No fertilizer was applied during any stage of plant growth (both at M_1 and M_2).

Screening of phenotypic (Macromutants) mutants

Macromutants were carefully screened at M_2 from seedling to maturity and the frequency of the mutants was estimated as per 100 plants in accordance with Gaul (1964). Chlorophyll mutants were classified after Blixt 1961. Seedling colors were laid down with reference to RAL COLOUR CHART (UK). The mutant trait(s) were confirmed at M_3 from selfed segregation of M_2 mutants.

Mutagenic efficiency and effectiveness

The efficiency and effectiveness of NPs, EMS and gamma irradiations were calculated from viable (total) mutation frequency (Walther 1969) as proposed by Konzak*et al.* (1965). The mutagenic efficiency was calculated as Mf/L, Mf/I and Mf/S and the effectiveness as (Mf/c) × t and Mf/Gy unit converted to kR (Mf = mutation frequency, L = lethality, I = injury, S = sterility, c = concentration, t = duration, Gy = gray unit and kR = kiloroentgen).

Meiosis

Meiotic analysis was performed in controls and in mutant plant types. For the purpose, floral buds (2 to 3 in each case) of suitable sizes were fixed (5 a.m. to 6.30 a.m.) in acetic alcohol 1:3 for overnight and preserved in 70% alcohol under refrigeration. Pollen mother cells (PMCs) and pollen grains obtained from anther squash preparations were stained in 2% acetocarmine solution. Fully stained pollen grains were considered fertile (Marks 1954). Scattered metaphase (MI) and anaphase I (AI) cells were only scored for analyses. Photomicrographs were taken from suitable preparations.

RESULT AND DISCUSSION

Mutation types andfrequencies

In comparison to normal trait(s) (Fig 1a), a total of 14 macromutant types (Table 1) are screened at M_2 mutagenized population (3357 plants scored). Out of 14 types, 9 in EMS, 6 in gamma irradiations, 13 in Cu-NPs and 14 in CdS-NPs are spotted. The mutants comprise of two non-viable types namely, *chloroxantha* (the plants dried at flowering stage: 51-57 days from sowing, bright yellowish green colored seedlings−Fig. 1b) and *viridis* (medium green colored seedlings, slow growing with reduced height, dried at flowering stage). The viable mutants are*sparsely arranged pinnae* I (associated with broad elongated pinnae−Fig. 1c) and II (Fig. 2d−e), *narrow pinnae*, *crumpled pinnae* (upward folding of pinnae to form cup like structure), feathery leaf, heterophyllous leaf (broad and narrow pinnae both present − Fig. 1f), cluster pinnae top, long petiole (Fig. 1g), thick stem (with associated bushy trait − Fig. 1h), dwarf, early flowering (37 to 39 days from sowing compared to 47 to 60 days in control plants) and synchronous maturity (Fig. 1i). Of all the mutants, synchronous maturity plant type is most significant as it will minimize seed loss at harvest. This mutant type appeared only in NPs treatments. The mutant *cluster pinnae top* is found specific to CdS-NPs treatments. The predominant mutant plant types recorded in all treated materials are *heterophyllous leaf*, *long petiole*, *sparsely arranged pinnae* I and II and *narrow pinnae*. No mutant was scored in controls. The mutant trait(s) in comparison to normal is presented in Table 2.

Across doses of treatments it seems that EMS has induced higher mutation frequency (viable: 9.95%, total: 11.44% than gamma irradiations (viable: 6.32%, total: 6.32%), Cu-NPs (viable: 5.95%, total: 6.44%) and CdS-NPs (viable: 3.92%, total: 4.26%). Higher mutation frequency in EMS than other treating agents may be due to low number of M_2 plant scored. Total failure of germination is recorded in 1.00% EMS, 3 and 6 h durations. Over the M_2 population, the macromutants (Table 1) occurred in the following order: EMS−*sparsely arranged pinnae* II >*chloroxantha* = *long petiole* = *dwarf*>*early*

flowering = narrow pinnae = sparsely arranged pinnae I >*heterophyllous leaf = thick stem*; gamma irradiation− *heterophyllous leaf*>*sparsely arranged pinnae* II >*long petiole*>*dwarf = narrow pinnae = sparsely arranged pinnae* I; Cu-NPs− *sparsely arranged pinnae* II>*heterophyllous leaf = synchronous maturity*>*dwarf*>*narrow pinnae = long petiole*>*sparsely arranged pinnae* I = *feathery leaf*>*viridis*>*chloroxantha = crumpled pinnae = thick stem = early flowering*; CdS-NPs−*sparsely arranged pinnae* II >*narrow pinnae* >*heterophyllous leaf* >*crumpled pinnae = cluster pinnae top = early flowering*>*sparsely arranged pinnae* I = *dwarf*>*viridis = feathery leaf = long petiole*>*thick stem*>*synchronous maturity*>*chloroxantha*. Apart from EMS (0.50%, 6 h−16.67%), maximum mutation (viable) frequency is mostly found in initial doses of gamma irradiations (25Gy− 8.82% and 50 Gy− 6.61%), Cu-NPs (0.50 µg/ml, 3 h−11.11%; 0.50 µg/ml, 6 h−16.37% and CdS-NPs (0.25 µg/ml, 3 h− 4.33%). The M_2 mutants segregated at M_3 in accordance with Mendelian patterns (data not given in the present communication) suggesting that the altered trait(s) than normal are genetically controlled.

Mutagenic effectiveness and efficiency

The mutagenic effectiveness (Tables 3 and 4) relates doses to mutation and is found to be maximum in EMS− 0.50%, 6 h, 25 Gy gamma irradiations, 0.50 µg/ml, 3 and 6 h Cu-NPs, 0.25µg/ml, 3 h CdS-NPs treatments.

On comparative basis it can be suggested that NPs are effective as mutagens like EMS and gamma irradiations. The mutagenic efficiency defined as the relation of number of mutational events to undesirable effects (lethality, injury and sterility) and is found to vary in relation to treating agents, and it seems that mostly threshold doses are efficient.

Meiotic analysis and pollen grains fertility

Meiotic chromosome behaviour is nearly normal and comparable in controls as well as in macromutants,PMCs regularly show 2n=12 chromosomes (Fig. 2 a−f) always. Controls show 6II formation in 100% meiocytesand it varies from 97.78% (*chloroxantha*) to 58.28% (*thick stem*) in mutants. Most of the univalents scored are rather due to precocious separation of chromosomes as mostly AI cells in mutants are balanced (92.45% to 100.0%). Rarely 1 to 2 laggards are observed at AI (Fig. 2g−h) and AII (Fig. 2i). Pollen grain fertility is assessed in controls (dry− 95.80%; bulk Cu− 95.79% and bulk CdS− 89.87%) as well as in mutants (70.25% in *viridis* to 89.81% in *heterophyllous leaf*).

The present investigation reveals the potentiality of Cu- and CdS-NPs in inducing macromutation in *N. Sativa*, which is corroborating to earlier reports in *Macrotylomauniflorum* (Halder*et al.* 2015 a, b). Thus, NPs inducing genetic changes can be explored for crop improvement.

Table 1. Macromutant types and frequency across doses in treatments.

Mutant types	Frequency (%)			
	EMS	Gamma irradiations	CdS-NPs	Cu-NPs
Chloroxantha	1.49	0.00	0.10	0.20
Viridis	0.00	0.00	0.24	0.29
Sparsely arranged pinnae I	0.99	0.55	0.34	0.39
Sparsely arranged pinnae II	1.99	1.65	0.63	1.37
Narrow pinnae	0.99	0.55	0.53	0.49
Crumpled pinnae	0.00	0.00	0.39	0.20
Feathery leaf	0.00	0.00	0.24	0.39
Heterophyllous leaf	0.50	2.20	0.48	0.78
Cluster pinnae top	0.00	0.00	0.39	0.00
Long petiole	1.49	0.82	0.24	0.49
Thick stem	0.50	0.00	0.19	0.20
Dwarf	1.49	0.55	0.34	0.68
Early flowering	0.99	0.00	0.39	0.20
Synchronous maturity	0.00	0.00	0.15	0.78
Total plants scored	201	364	2067	1025

Table 2. Mutant trait(s) in comparison to normal.

Mutants	Attributes	t-value	DF	Probability
Sparsely arranged pinnae I	Interpinnae distance Control-2.17±0.053	10.77	16	>0.001
(associated trait)	Mutant-3.49±0.110			
	Broad and elongated pinnae Length: control-2.22±0.029 mutant-3.06±0.040	18.78	18	>0.001
	Width:	5.52	16	>0.001

	control-0.23±0.017 mutant-0.36±0.018			
Sparsely arranged pinnae II	Interpinnae distance Control-2.16±0.047 mutant-2.83±0.128	4.90	16	>0.001
Narrow pinnae	Pinnae Length: control-2.22±0.290 mutant-0.76±0.037	29.68	18	>0.001
	Width: control-0.23±0.016 mutant-0.16±0.018	2.97	16	>0.01
Long petiole	Petiole length Control-5.62±0.084 Mutant-14.04±0.318	24.99	20	>0.001
Thick stem	Stem thickness Control-0.21±0.023 mutant-0.52±0.025	9.12	18	>0.001
Dwarf	Height Control-31.38±1.632 mutant-4.45±0.132	16.45	18	>0.001

Table 3. Mutagenic efficiency and effectiveness of EMS and gamma irradiation treatments in M_2 generation.

Treatments	(%/Gy)	Duration (h)	Per cent of control			Viable Mutation frequency (%) (Mf)	Mutagenic effectiveness (Mf/conc.)×duration (Mf/kR)	Mutagenic efficiency		
			Lethality (L)	Injury (I)	Sterility (S)			Mf/L	Mf/I	Mf/S
EMS	0.25	3	13.89	34.38	77.41	9.09	12.12	0.26	0.15	0.36
	0.50	3	13.89	50.91	75.51	8.77	5.84	0.11	0.05	0.07
	1.0	3	33.33	73.82	–	–	–	–	–	–
	0.25	6	8.33	41.87	75.04	6.67	2.73	0.74	0.16	0.18
	0.50	6	22.22	59.93	82.46	16.67	0.54	–	0.36	0.03
	1.0	6	94.44	94.16	–	–	–	–	–	–
Gamma irradiations	25	–	–	10.07	44.08	8.82	3.53	–	0.88	0.20
	50	–	22.22	6.91	67.14	6.61	1.76	0.30	0.96	0.09
	100	–	5.56	32.34	78.36	4.62	0.46	0.83	0.14	0.06
	200	–	52.78	62.84	100.00	–	–	–	–	–
	300	–	83.33	84.82	100.00	–	–	–	–	–

Table 4. Mutagenic efficiency and effectiveness of Cu- and CdS-NPs treatments in M_2 generation.

Treatments (%/Gy)		Duration (h)	Per cent of control			Viable Mutation frequency (%) (Mf)	Mutagenic effectiveness (Mf/conc.)× duration	Mutagenic efficiency		
			Lethality (L)	Injury (I)	Sterility (S)			Mf/L	Mf/I	Mf/S
CdS-NPs	0.25	3	16.67	28.51	11.91	4.33	5.77	0.26	0.15	0.36
	0.50	3	22.22	47.29	36.86	2.55	1.70	0.11	0.05	0.07
	1.0	3	8.33	0.49	17.01	5.42	1.81	0.65	11.06	0.32
	0.25	6	5.56	25.04	21.93	4.09	2.73	0.74	0.16	0.18
	0.50	6	–	4.55	40.68	1.62	0.54	–	0.36	0.03
	1.0	6	13.89	42.48	41.13	9.25	1.54	0.67	0.22	0.22
Cu-NPs	0.25	3	13.88	11.63	16.26	1.93	2.57	0.14	0.17	0.12
	0.50	3	2.77	32.09	25.35	11.11	7.41	4.01	0.35	0.44
	1.0	3	13.88	14.01	32.52	1.92	0.64	0.14	0.14	0.06
	0.25	6	5.55	6.31	31.47	5.70	3.80	1.03	1.03	0.18
	0.50	6	2.77	–	44.23	16.37	5.46	5.91	5.91	0.37
	1.0	6	5.55	10.07	43.53	2.88	0.48	0.52	0.52	0.06

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Figure plate 1 (a–i) showing control **(Fig. a)** and macromutant plant types **(b–i)** of *N. sativa*. **(Fig. a)** control; **(Fig. b)***chloroxantha*; **(Fig. c)***sparsely arranged pinnae* I-associated trait broad and elongated pinnae; **(Figs. d–e)***sparsely arranged pinnae* II; **(Fig. f)***heterophyllous leaf*; **(Fig. g)***long petiole*; **(Fig. h)***Thick stem* with bushy trait; **(Fig. i)** mutant showing synchronous maturity.

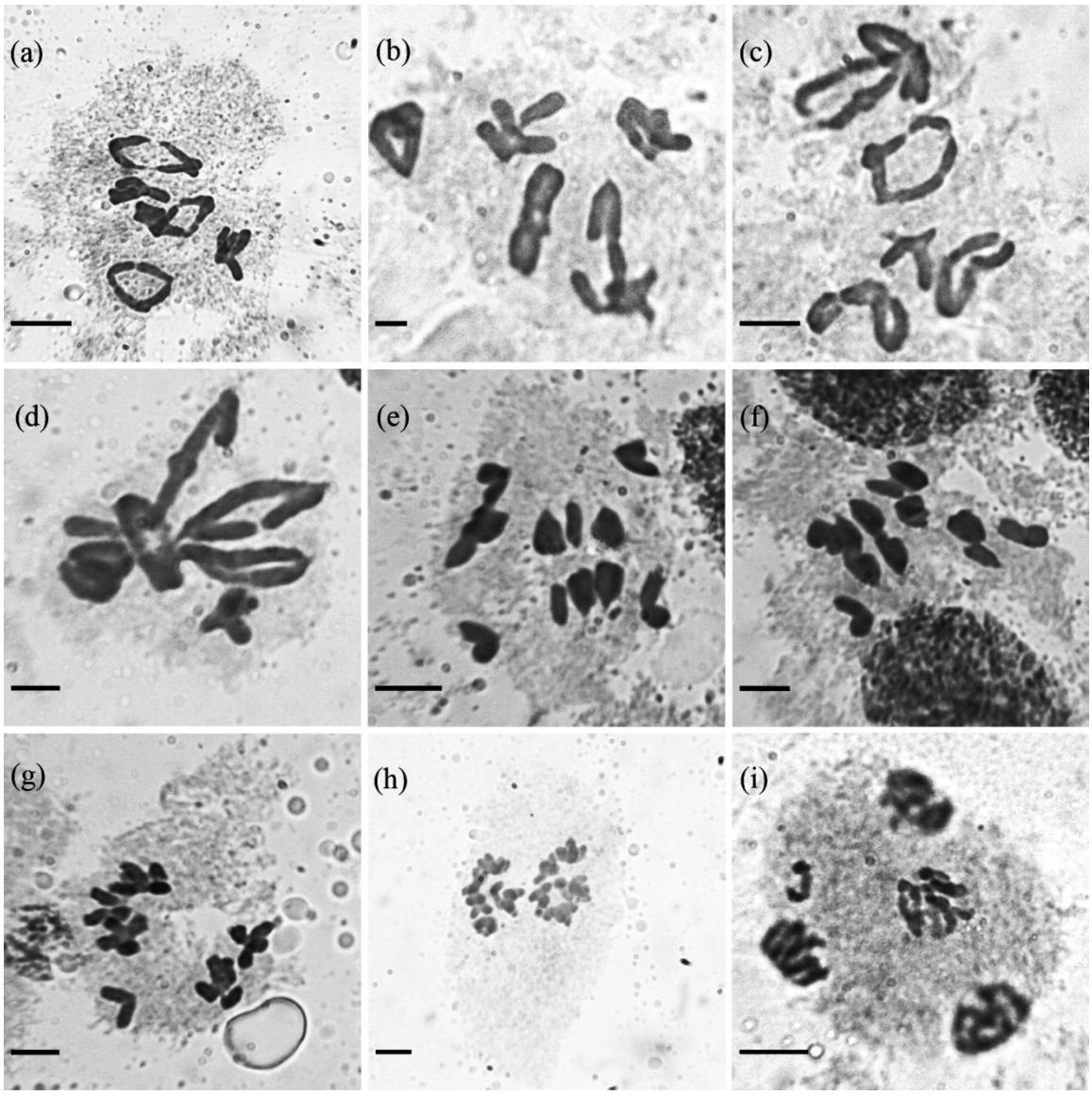

Figure plate 2 (a−i) showing meiosis configuration in mutants at MI **(a−f)**, AI **(g−h)** and AII **(i)**. **(Figs. a−d)** 6II (2n=12); **(Fig. e)** 1II+10I; **(Fig. f)** 2II+8I; **(Figs. g−h)** 5-1-6 separation of chromosomes; **(Fig. i)** a laggard. Scale bar=10µm.

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RESEARCH ARTICLE

AN AMINO ACID SEQUENCES BASED COMPUTATIONAL ANALYSIS OF ENZYME CYTIDYLATE KINASE

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Abstract: Computational analysis has been established for hypothetical study of amino acid sequences of the enzyme cytidylate kinase that derived from various programs and databases. Cytidylate kinase enzyme is widely distributed enzyme among bacteria and fungi. In the present study, thirteen full length amino acid sequences cytidylate kinase were retrieved, collected and subject to multiple sequence alignment (MSA), regular expression identification, domain identification, discovering individual amino acid composition, and construction of phylogenetic trees. Multiple sequence alignment revealed that three glycine, one lycine, one arginine and one valine were identically found in all the bacterial and fungal sources of cytidylate kinase. The two major sequence clusters were constructed by phylogenetic analysis. One cluster contains two species of fungi and six species of bacteria, where as other contain five species of only fungi. The amino acid composition results revealed that the average frequency of amino acid leucine is 9.29 % in fungi, where as alanine 13.61 % in bacteria. In addition, six unique motifs were also identified in the group analysis.

Keywords: Motif, Phylogentic analysis, Multiple sequence alignment, Cytidylate Kinase, Domain

INTRODUCTION

Kinases are a universal group of enzymes, which participate in a variety of cellular pathways. The name kinase is applied for enzymes, which catalyze the transfer of the terminal phosphate group from ATP to an acceptor that can be a small molecule, lipid, and protein substrate. The cellular and physiological roles of kinases are different (Cheek et al., 2002). Many kinases participate in signal transduction pathways, in which these enzymes are necessary components (Blenis, 1993). Other kinases are involved centrally in the metabolism of carbohydrates, lipids, nucleotides, amino acid residues, vitamins, and cofactors. Some kinases play roles in various other processes, such as gene regulation, muscle contraction, and antibiotic resistance. Their universal roles in cellular processes, kinases are the best-studied enzymes at the structural, biochemical, and cellular level. Although all kinases catalyze essentially the same phosphoryl transfer reaction, and display significant diversity in their structures, substrate specificity, and number of pathways in which they participate (Cheek et al., 2002).

Nucleoside monophosphate kinases (NMP kinases) are the key enzymes, which are involved in the metabolism of nucleotides. They act specifically on the various NMPs formed in *de novo* or salvage pathways of purine or pyrimidine nucleotides, by catalyzing the reversible transfer of a phosphoryl group from a nucleoside triphosphate to an NMP (Briozzo et al., 1998). CMP kinase is the key enzyme in the nucleotide metabolism that is connected to the family of nucleoside monophosphate kinase (NMK) (Leipe et al., 2003). Substrate specificity was studied on recombinant human UMP/CMP kinase (pyrimidine nucleoside polyphosphate kinase), which show that UMP and CMP (Verma et al., 2013) are far better substrates than dCMP (Liou et al., 2002).

Computational methods are used to analyze protein function that can be divided into three vast categories: sequence, expression and interaction based methods (Pellegrini, 2001). The success of computational approaches is used for solving important problems such as sequence alignment and comparisons (Altschul et al., 1990). The importance of this approach in research is used to annotate the proteome through functional and structural genomic efforts (Michalovich, 2002). Considering the above facts, a study of amino acid sequences of cytidylate kinase from different sources of organisms is really challenging. In the present study, the individual computational studies of amino acid sequences were performed, which were obtained from bacteria, fungi, and correlated them on the basis of some common feature.

MATERIAL AND METHOD

The full-length amino acid sequences of cytidylate kinase from bacteria and fungi were retrieved from protein databases available at NCBI (National Center for Biotechnology Information). The sequences were

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arranged as in bacterial and fungal profile, respectively. The multiple sequence alignment of the individual profiles was performed using CLUSTRALX (Bateman, 2007). Motifs were discovered in profiles using the expectation maximization approach implemented in multiple EM for motif elicitation server (Bailey et al., 2006). Further, the discovered motifs were used to search their protein family using Pfam at the sanger institute (Finn et al., 2010). The neighbor joining approach implemented in the MEGA (Molecular Evolutionary Genetics Analysis) program was employed for phylogenetic analysis (Tamura et al., 2011). The statistical reliability of the phylogenetic tree was tested by bootstrap analyses with 500 replications. MEGA program is also used for discovering individual amino acid composition.

RESULT AND DISCUSSION

Sequence retrieval and analysis

All the sequences belong to different families of bacteria and fungi were retrieved from genbank (National Center for Biotechnology Information) protein database and listed in Table 1 along with their accession number, organism name, family and source.

Table 1. list of retrieved sequences with their different sources

S.No.	Source	Name of Organisms	Family	Accession no.
1	Bacteria	*Streptococcus pneumoniae*	Streptococcaceae	KGI36288.1
2	Bacteria	*Staphylococcus aureus*	Staphylococcaceae	KII20889.1
3	Bacteria	*Salmonella enteric*	Enterobacteriaceae	CBY95005.1
4	Bacteria	*Escherichia coli*	Enterobacteriaceae	ACA78315.1
5	Bacteria	*Pseudomonas aeruginosa*	Pseudomonadaceae	KFL11511.1
6	Bacteria	*Mycobacterium tuberculosis*	Mycobacteriaceae	NP_216228.1
7	Fungi	*Trichoderma gamsii*	Hypocreaceae	KUE96443.1
8	Fungi	*Moesziomyces antarcticus*	Ustilaginaceae	XP_014653432.1
9	Fungi	*Fusarium langsethiae*	Nectriaceae	KPA47046.1
10	Fungi	*Rhizoctonia solani*	Ceratobasidiaceae	CUA68089.1
11	Fungi	*Puccinia sorghi*	Pucciniaceae	KNZ52000.1
12	Fungi	*Rhizopus microspores*	Mucoraceae	CEG68485.1
13	Fungi	*Mucor ambiguous*	Mucoraceae	GAN07890.1

Multiple sequence alignment

Multiple Sequence Alignment (MSA) showed the presence of some conserved residues in all the sequences from different sources while others were restricted only to their groups. Three glycine, one lycine, one arginine and one valine were found to be identically conserved residues in all analysed species in bacterial and fungal profile (Figure 1 and 2).

Conserved motif identification

Six conserved motifs were identified after the analysis of bacterial and fungal profile individually. Three conserved motifs were observed in bacterial and fungal profile (Table 2).

Conserveed motif family identification

The six identified conserved motifs were applied to their family identification in Pfam database using sequence search option. First three conserve motifs identified in bacterial profile belong to **Cytidylate_kin** domain family while last three conserved motifs, a single conserved motif identified in fungal profile belong to **AAA_17** domain family and in the Pfam entry of rest two conserved motifs no significant family was found (Table 2).

Table 2. Motif identified using MEME program and their pfam analysis using pfam database

S.No.	Motif	Width	Present in number of sequences	Family	Sources
1	[PG]G[IL][VI][AM]DGRD[IM]GTVV[FL]P DAP[LV]KIFL[DT]AS[ASV]EERA[EHR]R R[YM][LK]Q[LN]Q[AE]KG[FI][ES]V[DN]	49	6	Cytidylate_kin	Bacteria

	FE				
2	[PI]VI[AT]IDGP[AS]GAGK[GS]T[VL][AC]K[AR][LM]A[ER][AE]L[GQ]WH[LY]LD[ST]GA[MI]YR[AV]L[AT][LY]AAL[HK]H[GH]VD	48	6	Cytidylate_kin	Bacteria
3	L[LK]A[ED]I[KR][EA]RDDRD[SR]NR[AE]V[AS]PL[KV]PA[AD]DA[VL]VLD[ST]TG[LM]SIE[EQ]V[VI]EK[IA]L[AQ]Y[AV][ER][KQR][KR]	50	6	Cytidylate_kin	Bacteria
4	[SMP][KS][KD][ILV][FT][VR][IV][FA][VI][LD]G[GP]P[GA][AS]GK[GS]T[QT][CA][AK][RL]L[VA]E[DE][YL]GF[TV][HY][LI][SD][AS]G[DA][LM][LF]RA[EI][QT]Q[RK][ECP][GQ][SQ]QY	50	7	AAA_17	Fungi
5	[CT][PST]E[ED][VK][ML][LE][SKP]RL[LI]ERGKTSGR[ET]DDN[EAI]ESI[KR]KRF[RQ]TF[VAI][EQ]TSMPV	41	7	Pfam hit not found	Fungi
6	[FI]L[IVL]DGFPR[KER][ML][DE]QA[IVQ][KA]F[DE][EAR][ETS][VFI][CQV][PEIM][SAP][AKQSV][FL]VLF[FL]	29	7	Pfam hit not found	Fungi

Clustral analysis

Clustral analysis of bacterial profile

Clustral analysis of bacteria showed two major clusters as shown in Figure 4. Clustral A consist of four species namely *Salmonella enterica, Escherichia coli, Pseudomonas aeruginosa and Mycobacterium tuberculosis.* Clustral B consist of two species namely *Streptococcus pneumoniae* and *Staphylococcus aureus*, respectively.

Clustral analysis of fungal profile

Clustral analysis of fungi showed two major clusters as shown in Figure 3. Clustral A consist of five species namely *Moesziomyces antarcticus, Puccinia sorghi, Rhizoctonia solani, Fusarium langsethiae* and *Trichoderma gamsii*. Clustral B consist of two species namely *Rhizopus microsporus* and *Mucor ambiguous*, respectively.

Clustral analysis of joint bacterial and fungal profiles

Two major clusters were obtained by clustral analysis of joint bacterial and fungal profiles (Figure 5). Clustral A consists of eight species, which were further divided into two subclustral. Subclustral A contains three species of bacteria and two species of fungi. Subclustral B contains three species of bacteria and five species of fungi.

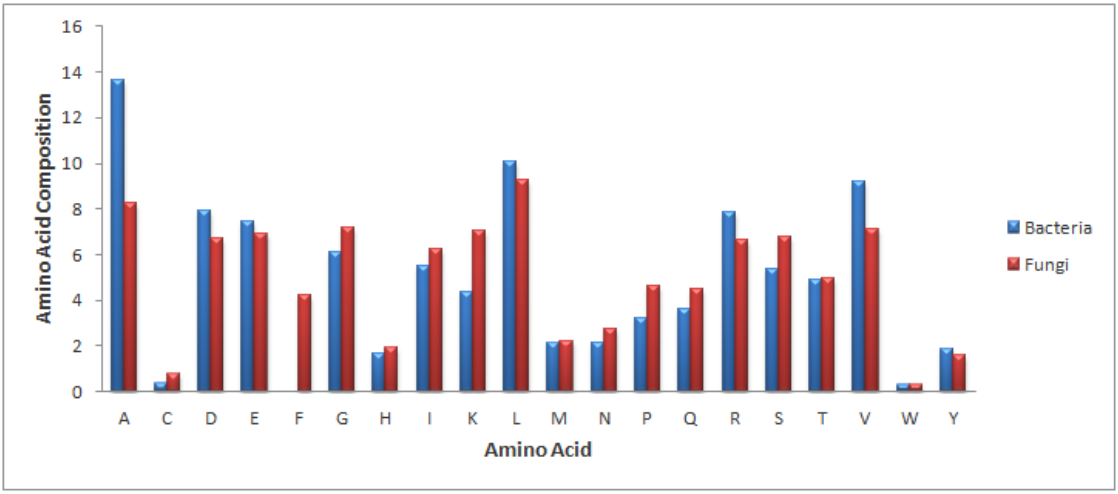

Fig. 1. Amino acid composition in different domain (Bacteria and Fungi)

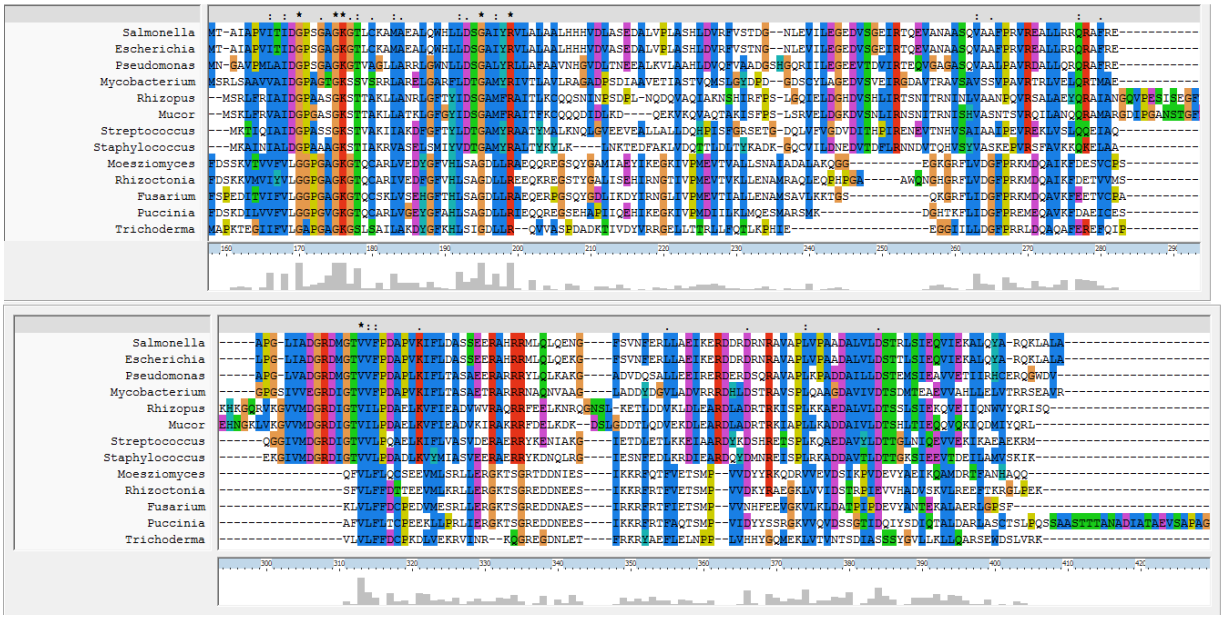

Fig. 2. The conservation study of enzyme cytidylate kinase between bacteria and fungi

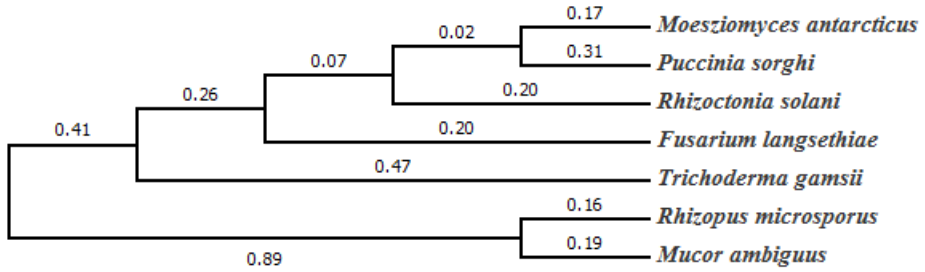

Fig. 3. Phylogenetic tree of fungal profile using neighbor joining method

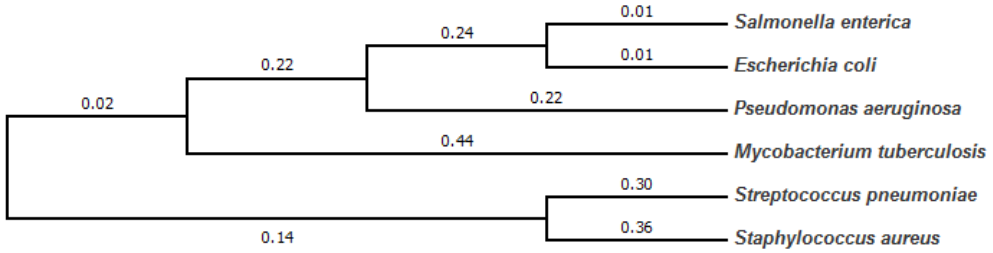

Fig. 4. Phylogenetic tree of bacterial profile using neighbor joining method

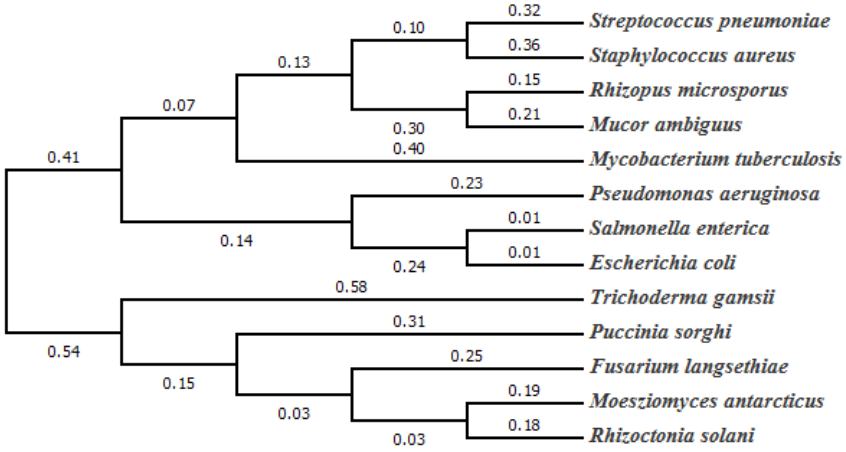

Fig. 5. Phylogenetic tree of joint profile of bacteria and fungi using neighbor joining method

CONCLUSION

Computational analysis of the cytidylate kinase sequences showed sequence-based similarities depending on their source organism. Three glycine, one valine, one lysine and one arginine residues were identically conserved in all analyzed species. These results suggested that the conserved amino acid residues have an important function in cytidylate kinase sequences. Six domains were identified in this research work, but three domains belong to Cytidylate_kin family that were found in all analyzed sequences of bacteria and fungi. These domains were found to be responsible for the functional activity of cytidylate kinase enzyme in different source organisms. These domains were conserved during the evolution of lower organism and their existence is important for the functional activity of this enzyme. In all species of bacteria and fungi an average frequency of amino acid leucine is 9.29 % in fungi, where as alanine 13.61 % in bacteria, which was higher in comparison to other amino acid average frequency. The amino acid alanine and leucine play an important role in the composition of cytidylate kinase. Two major sequences cluster were obtained by phylogenetic analysis. These phylogenetic analysis results suggested classification significance, which contributes the understanding of evolutionary relationship between the species at molecular level.

Competing interests

The authors declare no funding for this project, and no competing interests exits.

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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *ELAEOCARPUS GENITRUS* (RUDRAKSHA) SEEDS

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Abstract: In the present investigation phytochemical and antibacterial activity of *Elaeocarpusganitrus* (Rudraksha) seeds were studied with the methanolic and acetonic extract. The major phytochemical constituents screened were tannins, flavonoids, steroids, reducing compounds carbohydrates and alkaloids, alcohol and protein. It has been observed that maximum phytochemical compounds were present in methanolic and acetonic extract of *E. ganitrus*.The phytochemical screening was done to as certain the presence of bioactive components present in selected plant extract.Antibacterial activity in terms of minimum inhibitory concentration (MIC) of the extracts was studied with paper disc diffusion method and zone of inhibition was measured in mm. It has been observed that MIC was ranging from 11.25-21.25 mm for methanolic and 15.5-22mm for acetonic extract respectively. It is concluded that Rudkraksha seeds have many useful phytochemicals and possess significant antifungal/antibacterial activity.

Keywords: Phytochemical screening, Antibacterial activity, Rudraksha

INTRODUCTION

*Elaeocarpusganitrus*is commonly known as Rudraksha (Asolkar and Kakkar, 1992.). The word Rudraksha literally derived from two Sanskrit words 'rudra' a synonym for Lord Shiva and 'aksha' meaning eyes (Ramadurai, 2008). It is also called blueberry beads as beads are covered by an outer shell of blue color on fully ripening (Pandey and Das, 2004)). The seed is borne by several species of *Elaeocarpus*, with *Elaeocarpus ganitrus* being the principal species. Rudraksha was found in tropical and subtropical regions at the eminence ranging from seacoast to 2,000 meters above the sea level. Rudraksha cultivate in the area of the Gangetic plain in the foothills of the Himalayas to South-East Asia, Indonesia, New Guinea to Australia, Guam and Hawaii. Rudraksha tree flourish on mountains and hilly region of Nepal, Indonesia, Java, Sumatra and Burma.*Elaeocarpus* consists of about 12 genera and 350 species of tree (Gruissem and Jones, 2000).With the development of modern science, many scientists researched for evidences that support the ancient belief on the significance of Rudraksha(NISC, 2001**)**. All the scientists came up with the findings that reassured and confirmed the divine power of Rudraksha beads. Individual from every walk of life irrespective of caste, creed, religion, nationality or gender can use Rudraksha to gain maximum spiritual, physical and materialistic benefits.

The presence of alkaloids, carbohydrates, phenol, tannin, flavonoids, and protein was tested by a standard qualitative analysis (Middleton and Kandaswami, 1994). In Indian scenario the phytochemical screening and antibacterial activity of Rudrakshaseeds are not known or little work has been done. Therefore, the present study was carried out to fill the knowledge gap in this regard.

MATERIAL AND METHOD

Rudhraksha seeds were collected from the Nitza Biological Research Lab Hyderabad, Andhra Pradesh, India. Epicarp and endocarp powder of seeds were used for phytochemical analysis and antibacterial activity as per the method reported by Sharma and Sharma (2010).

Fig. 1. Rudraksha seeds and powder

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Phytochemical screening

Test for alkaloids (Mayer's Test)

Rudraksha endocarp (methanol and acetone extract) were treated with few drop of potassium mercurric iodide solution. Formation of a yellow colour precipitate indicates the presence of alkaloid. And Rudrasha epicarp (methanol and acetone extract) were treated with few drop of potassium mercurric iodide solution. Formation of a yellow colour precipitate indicates the presence of alkaloid.

Test for flavonoid

Rudraksha endocarp (methanol and acetone extract) were treated with sodium hydroxide solution. Formation of intense yellow colour indicate the absent of flavanoid.and Rudrasha epicarp (methanol and acetone extract) were treated with sodium hydroxide solution. Formation of intense yellow colour indicate the absent of flavonoid (Obasi*et al*., 2010; Audu*et al*., 2007).

Test for carbohydrates (Molisch's Test)

Rudraksha endocarp (methanol and acetone extract) were treated with few drop of alpha napthanol solution.not formation of violet ring at the junction indicate the absence of carbohydrates. And Rudrasha epicarp (methanol and acetone extract) were treated with few drop of alpha napthanol solution. Not formation of violet ring at the junction indicates the absence of carbohydrates.

Test for tannin

Rudraksha endocarp (methanol and acetone extract) were treated with 2% gelatin solution containing sodium chloride add the a few drops formation of white precipitation indicate the presence of tannin and rudrasha epicarp (methanol and acetone extract) were treated with 2% gelatin solution containing sodium chloride add the a few drops formation of white precipitation indicate the presence of tannin (Obasi*et al*., 2010; Audu *et al*., 2007).

Test for steroid

Rudraksha endocarp (methanol and acetone extract) were treated with acetic anhydride and 10 ml filtrate chloroform solution add the a few drops formation a ring of blue-green colour indicate the presence of steroid and Rudrasha epicarp (methanol and acetone extract) were treated with acetic anhydroide and 10 ml filtrate chloroform solution add the a few drops formation a ring of blue-green colour indicate the presence of steroid (Obasi*et al*., 2010,Audu*et al*., 2007).

Test for protein

Rudraksha endocarp (methanol and acetone extract) were treated with a few drops of concentrate nitric acid formation of yellow colour indicate the presence of protein .and Rudrasha epicarp (methanol and acetone extract) were treated with a few drops of concentrate nitric acid formation of yellow colour indicate the presence of protein (Obasi *et al*., 2010; Audu *et al*., 2007).

Test for phenol

Rudraksha endocarp (methanol and acetone extract) were treated with a few drops of ferric chloride solution formation of bluish black colour indicates the present of phenol. Rudraksha epicarp (methanol and acetone extract) were treated with a few drops of ferric chloride solution formation of bluish black colour indicates the present of phenol (Obasi *et al*., 2010; Audu *et al*., 2007).

Antibacterial activity

The agar well diffusion method was adopted to assess the antibacterial activity of the selected Rudraksha seeds (Baur *et al.,* 1966). Further 80 ml of nutrient agar media (NAM) was poured in four Petri plates. The Petri plates were labeled respectively and the media was allowed to solidified then 80µl of each of the bacterial suspension was inoculated on the surface of the solidified NAM.Then 80µl of extract was added to each well respectively. The plates were incubated at 37^{o}C for 18 to 24 hours. After the incubation the diameters of the growth inhibition zone were measured as describedby Singh and Nath (1999).

RESULT AND DISCUSSION

Phytochemical screening

The phytochemical screening of the rudrasha seeds (endocarp and epicarp) revealed that alkaloid is present in large amounts (Table no.1). And other classes present in small quantities: tannin, steroid, protein, phenol. However, the seed extracts tested negative for the presence of flavanoid and carbohydrates.

Table 1. Phytochemical constituents of Rudrasha seeds extracts

S. No.	Family of compound	Seed endocarp		Seed epicarp	
		Methanol	Acetone	Methanol	Acetone
1	Alkaloid	+ve	+ve	+ve	+ve
2	Flavanoid	-ve	-ve	-ve	-ve
3	Carbohydrates	-ve	-ve	-ve	-ve
4	Tannins	+ve	+ve	+ve	+ve

5	Steroid	+ve	+ve	+ve	+ve
6	Protein	+ve	+ve	+ve	+ve
7	Phenol	+ve	+ve	+ve	+ve

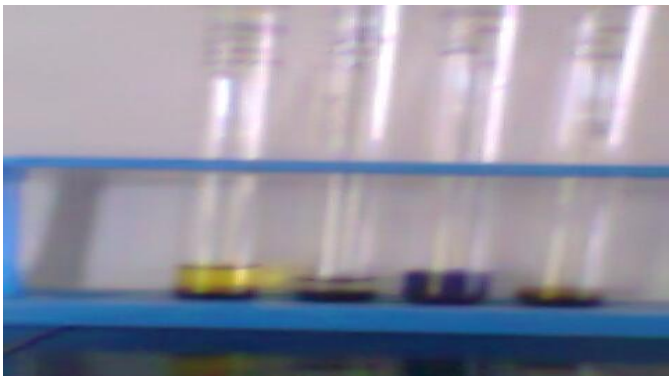

Fig 2. Phytochemical screening test

Antibacterial Activity

Out of Rudraksha seeds (endocarp and epicarp) tested for antibacterial activity, Rudraksha seeds (endocarp and epicarp) showed antibacterialactivity by inhibiting more microorganisms. The results of the antibacterial activity of Rudraksha seeds extracts tested agent microorganism by disk diffusion method are show in (table no-2) among the seeds extracts screened the methanol and acetone extracts. The Rudraksha seeds (endocarp and epicarp) showed in antibacterial activity but not antifungal activity.

Table 2. Antibacterial activity of Rudraksha seeds extract

Organisms	**Rudraksha Seed Endocarp/ Epicarp**	**Extract**	**Size of zone of inhibition**
(Bacteria) *Micrococcus*	Endocarp	Methanol	13.5mm
		Acetone	17.25mm
	Epicarp	Methanol	20.25mm
		Acetone	21.75mm
E. Coli	Endocarp	Methanol	12.75mm
		Acetone	15.5mm
	Epicarp	Methanol	20.75mm
		Acetone	21.5mm
Staphylococcus	Endocarp	Methanol	11.25mm
		Acetone	20.5mm
	Epicarp	Methanol	21.25mm
		Acetone	22mm

Fig. 2. Antibacterial activity endocarp (Staphylococcus)

Fig. 3. Measurment of zone (acetone epicarp)

CONCLUSION

The selected Rudraksha seeds (endocarp and epicarp) showed the source of the secondary metabolite i.e. alkaloid, carbohydrates, phenol, tannin, flavanoid, protein and amino acid. Rudrasha seeds play a vital role in preventing various diseases, the Antioxidant activities, anticancer activities, Antidiabetic activities, Antifungal activities, Antibacterial activities of the seeds are due to the presence of the above mentioned secondary metabolite. Rudrasha seeds are used for discovering and phytochemical screening and antibacterial activity which are very helpful for the manufacturing of new drugs. The phytochemical screening and antibacterial activity of the rudrasha seeds are also important and have commercial. Interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases.

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RESEARCH ARTICLE

ASSESSMENT OF YIELD LOSSES AND SCREENING OF PEA CULTIVARS FOR RESISTANCE TO ROOT ROT OF PEA CAUSED BY*FUSARIUM SOLANI* F.SP. *PISI*

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Abstract: Pea is an important legume crop widely cultivated throughout the world. Peas are grown in over 87 countries all over the world (Mcphee, 2003), providing food for humans and feed for domestic animals (Hargrove, 1986; Hulse, 1994; Patriarca *et al*., 2002).Although, peas have enormous nutritional qualities and have been considered to be the predominant export crop in world trade, representing about 40% of the total trade in pulses (Oram and Agcaoili, 1994).

Keywords: Pea, Legume crop, Root rot, Disease

INTRODUCTION

In India, Uttar Pradesh ranks first in the pea production with 1,532.39 thousand tonnes (Chandre*et al*., 2014). In India, pea is widely grown in area of 370 thousand million hectares, with production of 3517 thousand million tonnes and productivity 9.5 million tonnes/hectares. In Rajasthan state, pea crop has acreage of 3.6 thousand ha with annual production of 4.1 metric tonnes. The productivity in the state is 1.13 metric tonnes/ha (Anonymous, 2011). In Rajasthan pea is mainly cultivated as a vegetable and covering the maximum area and yielding maximum production in Jaipur district while productivity is higher in Bharatpur and Udaipur districts of Rajasthan (Anonymous, 1999).Among the soil borne diseases root rot of pea is a major soil borne disease in pea growing areas worldwide and is often considered to be the limiting factor in pea production (Shehata*et al*., 1983). Root rots are known to occur whenever peas are grown in the world (Fenwick, 1969; Hagedorn, 1976; Perssone*t al*., 1997). Root rot of pea caused by *Fusariumsolani*f.sp. *pisi*is a disease of economic importance causing considerable damage to the crop and remains to be challenging task in terms of management(Maheshwariand Jhooty,1983; Tu, 1986; Kumar and Dubey, 2000).Root rot of pea caused by *Fusariumsolani*remained a major threat to successful cultivation of pea and resulted in reduced crop yield and ultimately caused heavy economic losses. It caused severe damage at all stages of crop growth, and up to 97% yield losses had been reported by Sen and Majumdar (1974).

Among the soil borne diseases root rot of pea is a major soil borne disease in pea growing areas worldwide and is often considered to be the limiting factor in pea production (Shehata *et al*., 1983).Root rot may start when the plant is in the pre or post emergence seedling stage. Death soon follows as early infections, resulting in a poor crop stand. Root decay generally begins on the finer feeder roots and progresses gradually to the main tap root of the plant. In some cases all roots are destroyed, leaving only remnants below the attachment of the seed. Root rot of pea is characterized by the cortical decay and a brilliant red discoloration of vascular tissues in the root (Lin *et al*., 1984).

The underground part of pea plant is damaged by the fungus. On underground stem reddish brown sunken lesions are formed. The root system may be completely decayed and the plant has poor standing. Vascular reddish discoloration is commonly observed in diseased plants. Symptoms consist of poor growth, yellowing and finally wilting of leaves (Singh, 1999). Among the fungal diseases, the root rot caused by *Fusariumsolani*f.sp.*pisi*remains to be challenging task in terms of management. Therefore, integrated management strategy is the better solution to maintain plant health. These strategies include minimum use of chemical for checking the pathogen population, encouragement of beneficial biological agent to reduce pathogen inoculums, modification of cultural practices and use of resistant varieties (Bendre and Barhate, 1998).

MATERIAL AND METHOD

Assessment of Yield Losses

The losses caused by a disease vary with host pathogen interaction and the disease severity. Field trials were conducted in two consecutive during rabi (2010) and (2011) to assess germination percentage, root rot incidence, green pod yield and reduction in pod yield under different disease severity (generated by different inoculums densities). This region has a semi-arid climate. The soil of the experimental fields is sandy-loam in texture, slightly alkaline (pH 7.9), having low organic carbon (0.42) and electrical conductivity (0.85 dSm^{-1}). The experiment was conducted using a local cultivar at three inoculums densities *viz*., 50g/plot, 100g/plot and 150g/plot. Un inoculated plots were maintained as control. The

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inoculated plots were compared with un-inoculated plots. The seeds were sown in 3x2 m plots, keeping ten rows(30 cm) and 20 plants in each row, at 10 cm plant distance. Recommended agronomical practices for fertilizers (N-80, P-40 & K-40 Kg ha-1) and weed management**,** pre- germination spray of Atrazine at 0.5% and mechanical removal were followed, but no fungicide was used in this trial. The inoculum was multiplied on corn meal sand (2:1) medium. The inoculations were done in the late evening, followed by heavy irrigation to provide adequate moisture for infection. Observation for seed germination wererecorded at 15 days after sowing.Theobservation for plant mortality were recorded 90 days after sowing.

Screening of pea cultivars

Eleven cultivars were evaluated under artificial inoculation conditions using soil inoculation technique of spore cum mycelia (50 gm/ row) of *F. solani*f.sp. *pisi*causing root rot of pea. Cultivars/germplasm*viz.*, KashiSamrath, VRP-22, VRP-6, VRP-7, VRP-5, Pea IP-3, Azad P-1, Pea KS-210, Pea KS- 205, Pea VP-433 and Local cultivar were evaluated against root rot pathogen in artificially inoculated sick plots. These cultivars were procured from IIVR, Varanasi. Seeds were sown in a single row each of 5 m length and maintaining row and plant to plant distance as 30x10 cm respectively. In this screening technique a root rot susceptible check was sown intermittently after every two test entries so as to monitor the disease pressure.The observations for germination percentage,% disease incidence c.f.u. of *F. solani* in rhizosphere& Green pod yield were recorded.

RESULT AND DISCUSSION

Assessment of Yield Losses

To determine the losses caused by root rot in pea, field trials were conducted in the Experimental fields of RCA, MPUAT, for two consecutive years *rabi* (2010-11) and (2011-12), by creating different disease levels through varied inoculum densities of *F. solani*f.sp. *pisi*on pea local variety and compared with uninoculated protected plots. Observations on percent seed germination, percent plant mortality, green pod yield and per cent yield losses were recorded. The seed germination was recorded 15 days after sowing and plant mortality was recorded 90 days after sowing.The data are presented in Table 1.

Table 1. Effect of inoculum load of *Fusariumsolani*f.sp.*pisi* on per cent seed germination, per cent plant mortality and per cent yield loss during *rabi* season (2010-11) and (2011-12)

S. No.	Treatments (Inoculum loads per plot in g.)	Seed germination* (%)			Plant mortality* (%)			Green Pod yield* Kg/ plot			Yield losses* (%)		
		2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled
1.	50	85.77 (67.88)	87.40 (31.28)	86.59 (49.70)	20.69 (27.04)	19.53 (31.28)	20.11 (29.28)	3.35	3.38	3.37	24.73 (30.14)	21.12 (27.90)	22.92 (28.95)
2.	100	74.44 (59.26)	72.03 (32.82)	73.23 (46.12)	31.86 (34.80)	32.17 (32.82)	32.02 (33.41)	2.11	2.13	2.12	52.40 (46.60)	50.40 (45.34)	51.40 (45.95)
3.	150	68.51 (55.87)	70.55 (31.11)	69.53 (43.51)	47.70 (43.68)	45.97 (31.11)	46.84 (37.42)	1.11	1.23	1.17	74.88 (59.94)	71.09 (57.59)	72.98 (58.78)
4.	Un inoculated control	95.36 (78.16)	94.62 (30.73)	94.99 (55.60)	6.38 (14.63)	8.62 (30.73)	7.50 (24.11)	4.45	4.30	4.38	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
SEm±		1.22	2.15	1.23	0.49	2.15	1.10	0.11	0.05	0.06	1.42	0.73	0.80
CD at 5%		3.75	6.61	3.60	1.50	6.61	3.21	0.34	0.17	0.18	4.37	2.26	2.33
CV%		3.73	13.33	7.16	3.25	13.33	10.02	7.90	3.89	6.22	8.30	4.48	6.75

* Mean of three replications

Figures in parenthese are arcsine √ per cent angular transformed values.

c.f.u of inoculum prepared in lab is 3 x 10^6

Pooled data of both the years (2010-11 and 2011-12) revealed that among the different inoculum loads used, the highest germination 86.59% recorded in 50 g inoculum/plot (T_1), followed by 73.23% germination with 100 g inoculum/plot (T_2), 69.53% germination with 150 g inoculum/plot (T_3). The highest germination 94.99% was observed in uninoculated control plots (T_4). Mean mortality recorded in uninoculated plots (T_4) was 7.50%. Among the inoculated plots highest 46.84% mortality was recorded with 150 g inoculum/plot (T_3) followed by 32.02% mortality with 100 g inoculum/plot (T_2) and 20.11% mortality with 50 g inoculum/plot (T_1).The uninoculated control plots (T_4) yielded 4.38 kg/plot green pod yield, those inoculated with 50 g inoculums/plot (T_1) yielded 3.37 kg/plot green pod yield, those with 100 g inoculum/plot (T_2) yielded 2.12 kg/plot green pod yield and those with 150 g inoculum/plot (T_3) yielded 1.17 kg/plot green pod yield.The highest yield loss (72.98%) among the

inoculated plots was recorded with 150 g inoculum/plot (T_3) followed by 51.40% yield loss with 100 g inoculum/plot (T_2), 22.92% yield loss with 50 g inoculum/plot (T_1).

Field experiments were conducted for two consecutive years to estimate the losses caused by root rot of pea with disease generated through different inoculum densities on local variety of pea. Results showed that the disease severity increased with increasing of inoculum loads. A significant reduction in green pod yield at all the severity levels was observed as compared to the control. Pooled data revealed that lowest germination per cent (69.53%) with maximum plant mortality (46.84%) and highest yield loss (72.98%) was observed in plots inoculated with higher inoculum load i.e. 150 gm/plot whereas maximum germination (86.59%) with minimum plant mortality (20.11%) and lowest yield loss (22.92%) was observed in plots inoculated with low inoculums load i.e. 50 gm/plot.These observations suggest that root rot of pea has good potential of damaging the crops and may become limiting factor in realization of good yield.

Screening of pea cultivars

Eleven pea cultivar were screened to find out the source of resistance against *F. solani*f.sp. *pisi* causing root rot of pea. The experiment was conducted in field using the varieties KashiSamrath, VRP-22, VRP-6, VRP-7, VRP-5, Pea IP-3, Azad P-1, Pea KS-210, Pea KS- 205, Pea VP-433 and Local cultivar in the field where *F. solani*f.sp. *pisi*used for soil inoculation. The experiment was conducted in *rabi*season (2010-11) and (2011-12). Observations on germination percentage, per cent disease incidence, green pod yield and c.f.u. of *F. solani*f.sp.*pisi*were recorded and presented in Table 2 . The seed germination was recorded 15 days after sowing and plant mortality was recorded 90 days after sowing.

Table 2. Screening of pea cultivars for resistance to root rot pathogen in field during *rabi* season 2010-11 and 2011-12

S.No.	Cultivar/ Germplasm	Germination* (%)			Plant mortality* (%)			Green pod yield* Kg/ 5m row			Cfu of *F. solani*f.sp. *pisi* x$10^4$90 DAS		
		2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled	2010-11	2011-12	Pooled
1.	Kashisamrath	82.66 (65.40)	82 (64.92)	82.33 (65.16)	46.46 (42.96)	50.59 (45.34)	48.53 (44.15)	0.81	0.71	0.76	7.8	9.3	8.55
2.	VRP- 22	89.33 (70.98)	90.00 (71.62)	89.67 (71.30)	35.58 (36.61)	38.72 (38.45)	37.15 (37.53)	1.24	1.20	1.22	5.0	6.5	5.75
3.	VRP- 6	92.66 (74.53)	92.00 (73.65)	92.33 (74.09)	32.11 (34.50)	33.15 (35.13)	32.63 (34.82)	1.34	1.31	1.33	3.1	3.9	3.50
4.	VRP- 7	94 (75.95)	94.66 (76.70)	94.33 (76.33)	26.27 (30.80)	29.56 (32.93)	27.92 (31.86)	1.71	1.63	1.68	2.3	3.5	2.90
5.	VRP- 5	91.33 (73.25)	90.66 (72.23)	91.00 (72.74)	34.29 (35.83)	36.27 (37.02)	35.28 (36.43)	1.27	1.25	1.26	4.5	5.0	4.75
6.	Pea IP-3	88.65 (70.52)	88.00 (69.77)	88.33 (70.15)	39.07 (38.68)	40.43 (39.48)	39.75 (39.08)	1.14	1.10	1.12	5.5	6.8	6.15
7.	Azad pea- 1	81.30 (64.39)	80.66 (63.92)	80.98 (64.16)	54.04 (47.32)	57.20 (49.21)	55.62 (48.27)	0.64	0.58	0.61	8.1	10.5	9.30
8.	Pea KS-210	87.33 (69.34)	86.66 (68.73)	87.00 (69.04)	41.14 (39.89)	41.45 (40.08)	41.29 (40.12)	1.04	1.05	1.05	5.9	7.5	6.72
9.	Pea KS-205	85.33 (67.55)	86.00 (68.06)	85.67 (67.81)	41.40 (40.05)	43.33 (41.16)	42.36 (40.35)	0.96	0.90	0.93	6.3	7.9	7.10
10.	Pea VP-433	84.66 (67.02)	84.00 (66.45)	84.33 (66.73)	41.62 (40.17)	47.38 (43.51)	44.50 (41.78)	0.90	0.79	0.85	6.8	8.2	7.50
11.	Local	79.99 (63.44)	78.00 (62.04)	79.00 (62.74)	60.30 (50.99)	59.14 (50.29)	59.72 (50.64)	0.47	0.48	0.48	8.9	10.9	9.90
	SEm±	1.45	0.89	0.85	1.03	1.44	0.89	0.04	0.045	0.03	0.27	0.29	0.20
	CD 5%	4.27	2.63	2.43	3.05	4.25	2.53	0.13	0.13	0.09	0.80	0.87	0.57
	CV %	4.17	2.59	3.48	5.19	7.01	6.20	8.34	9.03	8.68	9.24	8.08	8.60

* Mean of three replications

**Initial Population of *F. solani*f.sp. p*isi* 1.70 x 10^4c.f.u./g soil

Figures in parenthesearearcsine$\sqrt{}$ per cent angular transformed values. c.f.u of inoculum prepared in lab is 3 x 10^6

Pooled data of two seasons revealed among the varieties, the lowest germination (79.00%) was recorded with local pea variety followed by variety Azad Pea-1 with 80.98% germination. KashiSamrath with 82.33 % germination, Pea VP-433 with 84.33% germination , Pea KS-205 with 85.67% germination, Pea KS-210 showed 87.00% germination, Pea IP-3 with 88.33% germination, VRP-22 with 89.67% germination, VRP-5 with 91.00% germination, VRP-6 with 92.33% germination and the highest germination 94.33% was recorded with VRP-7. Among the varieties tested, the highest mortality 59.72% was recorded with local pea variety, followed by 55.62% mortality in Azad Pea-1, 48.53% mortality in KashiSamrath, 44.50% mortality in Pea VP-433, 42.36% mortality in Pea KS-205, 41.29% mortality in Pea KS-210, 39.75% mortality in Pea IP-3, 37.15% mortality in VRP-22, 35.28% mortality in VRP-5 and 32.63% mortality in VRP-6. The lowest mortality 27.92% was recorded with VRP-7. Highest green pod yield 1.68 kg/ row among different varieties reported in VRP-7 as compared to local pea which yielded lowest yield 0.48 kg/row. With variety VRP-6 1.33 kg/row green pod yield was reported followed by 1.26 kg/row green pod yield from VRP-5, 1.22 kg/row green pod yield from VRP-22, 1.12 kg/row green pod yield from Pea IP-3, 1.05 kg/row green pod yield from Pea KS-210, 0.93 kg/row green pod yield from Pea KS-205, 0.85 kg/row green pod yield from Pea VP-433, 0.76 kg/row green pod yield from KashiSamrath, 0.61 kg/row green pod yield from Azad Pea-1.

Pooled data revealed that the lowest population density $2.90x10^4$ cfu/g soil was observed in rhizosphere of variety VRP-7 as compared to rhizosphere of local pea variety where highest population density of pathogen $9.90x10^4$cfu/g soil was observed. In VRP-6 rhizosphere $3.50x10^4$cfu/g soil was reported followed by $4.75x10^4$cfu/g soil in VRP-5, $5.75x10^4$cfu/g soil in VRP-22, $6.15x10^4$cfu/g soil in Pea IP-3, $6.72x10^4$cfu/g soil in Pea KS-210, $7.10x10^4$cfu/g soil in Pea KS-205, $7.50x10^4$cfu/g soil in Pea VP- 433 and $8.55x10^4$cfu/g soil in KashiSamrath and $9.30x10^4$cfu/g soil in Azad Pea-1.

The management of the diseases through host plant resistance is considered as a dependable choice in all the crop improvement programmes. Utilization of resistance cultivars in farming is simple, effective and economical method for management of the diseases. The resistant cultivars reduce the cost, time and energy when compared to the other methods of disease management. Screening was done taking eleven pea cultivars with inoculation of pathogen using soil inoculation technique of spore cum mycelia of *Fusariumsolanif*.sp. *pisi* .The result showed that out of eleven cultivars tested one cultivar namely VRP-7 was found moderately resistant, four cultivars namely VRP-6, VRP-5, VRP-22 and Pea IP-3 were found moderately susceptible whereas six cultivars namely Pea KS-210, Pea KS-205, Pea VP-433, Kashisamrath, Azad pea-1 and local pea cultivar were found susceptible. Thus, among the eleven cultivars tested none of the cultivar was found free to the disease infection. The pooled c.f.u. of *Fusariumsolanif*.sp.*pisi* in the rhizosphere area of all cultivars by using serial dilution technique at the time of sowing and 90 DAS. It was observed that c.f.u. of pathogen in the rhizosphere of moderately resistant cultivar was $2.90x10^4$ whereas in moderately susceptible cultivars it ranged from $3.50x10^4$ to $6.15x10^4$ and in susceptible cultivars it ranged from $6.72x10^4$ to $9.90x10^4$.

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RESEARCH ARTICLE

EFFECT OF CROP ESTABLISHMENT METHOD AND IRRIGATION SCHEDULES ON PRODUCTIVITY AND WATER USE OF WHEAT

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Abstract: A field experiment was conducted during 2014-15and 2015-16 at Meerut, Uttar Pradesh. The grain yield (46.52; 47.63 and 44.01 and 44.88 q ha^{-1}), straw (60.57; 61.55 and 59.94; 102.75 q ha^{-1}) biological yield (107.09; 109.40 and 102.75; 104.82 q ha^{-1}) was and harvest index (43.39; 43.49 and 42.53; 42.77) significantly higher in $B_{90–4}$ and 4 cm irrigation at IW/CPE 0.8 during both the year. Physiological traits, yield attributes and yields were significantly influenced by land configuration and wheat irrigation schedules. In land configuration systems, $B_{90–4}$and 4 cm irrigation at IW/CPE 1.2 displayed significantly higher water use efficiency (2.53; 2.51 and 2.19; 2.18 kg m^{-3}) compared with other treatments. However irrigation schedules × land configurationinteraction was significant for yield attributes grain, straw and biological yield except 1000 grain weight.

Keywords: Land configuration, Irrigation schedules IW/CPE, Water use efficiency

INTRODUCTION

Wheat a major cereal crop is being cultivated in the country. The main reasons for its productivity are poor crop establishment and improper scheduling of irrigation. Amongst the other agronomic practices proper crop establishment method may considerably increase the production of wheat up to some extent. Ideal planting geometry is important for better and efficient utilization of plant growth resources get the optimum productivity of wheat. It is also well know fact that water management is one of the major factors responsible for achieving better harvest in crop production. Both crop establishment method and irrigation schedule are major causes of yield reduction in wheat, which also affect its water use efficiency. Farmers are always interested in getting higher yield which could not be possible without better crop management, good stand establishment and optimum utilization of resources. Crop production is influenced by its establishment and plant vigor representing the key factors towards crop development (Amanullah*et al.,* 2009).

To increase the water productivity of wheat, CYMMIT introduced a planting pattern termed as furrow irrigated raised bed planting system in Mexico. The adoption of the system rose from 6% of farmers in 1981 to 75% in 1994 in high-yielding irrigated wheat-growing areas of northwestern Mexico (Sayre and Hobbs 2004). In this system, the crop is planted on the top of beds and irrigation water is applied in furrows. The width of the bed and furrows commonly used are 40–45 and 25–30 cm, respectively, and the bed height is 15 cm– 20 cm. Inspired by the success of irrigated maize–wheat on permanent raised beds in Mexico, furrow irrigated raised bed planting system was introduced in Indo-Gangetic Plains in the mid-1990s for wheat (Sayre and Hobbs 2004). Even after 2 decades of its introduction and promotion, a few farmers preferred bed planting over the conventional flat planting system. This was mainly due to lack of yield advantage in furrow irrigated raised bed planting system over flat planting system. Farmers can easily respond to this technology if efforts are diverted to demonstrate yield differences between flat and bed planting systems either by modifying bed configuration or crop rows planted on the top of the bed or selection of suitable cultivars. Keeping in view the above points, a study was conducted to compare crop establishment method especially in different bed size configurations and rows planted on the top of the bed with flat planting in wheat. Different crop establishment method were assessed for wheat productivity and water saving.

MATERIAL AND METHOD

Experimental site

The field experiment was established in 2014 at SardarVallabhbhai Patel University of Agriculture &Technology, Meerut research farm (29^0 04', N latitude and77^0 42' 'E longitude a height of 237m above mean sea level) U.P., India. The region has a semi-arid sub-tropical climate with an average annual temperature of 16.8^0C. The highest mean monthly temperature (38.9^0C) is recorded in May, and the lowest mean monthly temperature (4.5^0C) is recorded in January. The average annual rainfall is about 665 to 726 mm (constituting 44% of pan evaporation) of which about 80% is received during the monsoon period. The predominant soil at the experimental site is classified as TypicUstochrept. Soil samples for 0–20 cm depth at the site were collected and tested prior to applying treatments and

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the basic properties were non-saline (EC 0.42 dS m^{-1}) but mild alkaline in reaction (pH 7.98). The soil initially had 4.1 g kg^{-1} of SOC and 1.29 g kg^{-1} of total N (TN), 1.23 g kg^{-1} of total phosphorus, 17.63 g kg^{-1} of total potassium, 224 mg kg^{-1} of available N, 4.0 mg kg^{-1} of available phosphorus, and 97 mg kg^{-1} of available potassium.

Experimental design and management

A detailed description of crop establishment methods are necessary to compare the influence of land configuration practices on environmental performance (Derpsch et al., 2014).Six crop establishment methodsB_1- 75 cm bed, 2 rows ($B_{75–2}$); B_2-, 75 cm bed, 3 rows ($B_{75–3}$) ; B_3- 90 cm bed, 2 rows ($B_{90–2}$) ; B_4- 90 cm bed, 3 rows ($B_{90–3}$), B_5- 90 cm bed, 4 rows ($B_{90–4}$); B_6- Flat planting , rows 22.5 cm apartin main plots and three irrigation schedule practices were I_1-4 cm irrigation at IW/CPE 0.8; I_2-5 cm irrigation at IW/CPE 1.0; I_3- 6 cm irrigation at IW/CPE 1.2allotted to sub-plots in a split-plot design andreplicated thrice. The gross and net plot sizes were 7.0 m×24.5 m and 6.0 m×3.5 m, respectively and treatments were superimposed in the same plot every year to study the cumulative effect of treatments.

Preparation of furrow irrigated raised beds

At the beginning of the experiment soil was tilled by harrowing and plowings followed by one field leveling with a wooden plank, and raised beds were made using a tractor-drawn multi crop zero till cum raised bed planter with inclined plate seed metering devices. The dimension of the raised beds were 45 and 60 cm wide (top of the bed) x 18 cm height x 30 cm furrow width (at top) and the spacing from center of the furrow to another center of the furrow was kept at 75 and 90 cm. In furrow irrigated raised bed planting system, the crop was planted on the top of beds in bed configurations of 45 cm bed and 60 cm bed.

Preparation of Conventional tillage

After the rice harvest, following the conventional practice of two harrowing, two ploughing (using a cultivator) and one planking (using a wooden plank) that followed pre-sowing irrigation and wheat was seeded in flat planting, a uniform row-to-row distance of 22.5 cm was maintained. Using a seed drill with a dry-fertilizer attachment.

Nutrient application

Plant nutrients were applied as per the state recommendations for wheat (N_{120}+ P_{60} + K_{40}). Urea, di-ammonium phosphate and muriate of potash, were placed in band in seed rows at the time of sowing using zero till cum raised beds planter with inclined plate metering device. The remaining N was broadcasted with dry urea in two equal splits of 30 kg N ha^{-1}, (N_{30}) at crown root initiation (CRI) and the flag leaf initiation (FLI) crop growth stages.

Sowing techniques

Wheat cultivar DBW-17 was shown on November 2015 and 2016 using 80 kg ha^{-1} for raised beds and 100 kg seed ha^{-1} for flat planting was done using zero till cum raised beds planter with inclined plate metering device.

Weed management

The crop was maintained with weed free using following practices. Weeds were controlled by spraying of herbicide Sulfosulfuron + Metsulfuron (Total) 35 g a.i. ha^{-1} and applied uniformly in standing crop to control the weeds at 30-45 DAS. To check the weed growth, one inter culture operation was done during 2014-15 and 2015-16 eight weeks after sowing with the help of manual weeding.

Irrigation scheduling

Measured quantity of irrigation water was applied to the plots as per the irrigation schedule. For measuring irrigation water, volume method was used. Irrigations were scheduled on IW: CPE ratio in individual treatments. The source of irrigation water was Tube well with good quality water for irrigation.

Water application and measurements

Irrigation water was applied using polyvinyl chloride pipes of 15-cm diameter and the amount of water applied to each plot was measured using a water meter (Dasmesh Co., India). The quantity of water applied and the depth of irrigation was computed using the following equations:

Quantity of water applied (L) = $F \times t$ ...(1)

Depth of water applied (cm) = L /A /1000 ...(2)

Where F is flow rate (L/s), t is time (s) taken during each irrigation and A is area of the plot (m^2). Rainfall data was recorded using a rain gauge installed within the meteorological station. The total amount of water (input water) applied was computed as the sum of water received through irrigation (I) and rainfall (R).Water productivity (WP_{I+R}) (kg/m^3) was computed as follows (Humphreys *et al,* 2008)

WP_{I+R} =Grain yield/(Irrigation water applied (I) + Rainfall received by the crop (R)). ...(3)

Water use studies

Soil moisture content was measure at seeding, and before and after each irrigation on the top of the ridge and furrow in furrow irrigated raised bed planting system and between the 2 rows in flat planting by using neutron moisture meter. Water saving (WS) was calculated as:

$$WS = (Q_F - Q_B)/Q_F \times 100,$$

Where Q_F and Q_B are quantity of water applied in flat planting and furrow irrigated raised bed planting system, respectively. The soil moisture data would be utilized to calculate the consumptive use.

RESULT AND DISCUSSION

Yield attributes

Data on various yields attributing characters viz. spike length, number of spikelet's spike^{-1}, number of grains spike^{-1}, and test weight, as influenced by land configuration and different irrigation schedules are presented in (Table 1) revealed that $B_{75\text{-}2}$ land configuration significantly higher spike length over other treatments. The number of spikelet's spike^{-1}, number of grains spike^{-1}, and test weight higher with $B_{90\text{-}2}$ as compare to remaining treatments during the year of study. The number of grains pike^{-1} was higher in 90 cm than 75 cm beds and flat planting. The irrigation scheduling 4 cm irrigation at IW/CPE 0.8 recorded significantly values for all the above yield attributes as compare to other irrigation schedules. Stimulated vegetative growth of wheat on account of adequate and prolonged supply of water in treatment manifested itself in increased spike length, number of spikelet's spike^{-1}, number of grains spike^{-1}, and test weight(Jat and singh 2003; Maurya and singh 2008;).

Interaction effects between irrigation schedules and land configuration in relation to spike length, number of spikelet's spike^{-1}, number of grains spike^{-1}, and test weight were significant (Table 1). The magnitude of increase in spike length due to improvement in moisture supply by irrigation with furrow irrigated raised beds was observed under IW/CPE 0.8 (I_1) irrigation schedules with $B_{90\text{-}2}$ land configuration as compared to other treatments combination during 2014-15 and 2015-16, respectively.

Table 1. Effect of land configuration and irrigation schedules on yield attributes

Treatment	Spike length (cm)		No. of Spikelet's spike^{-1}		No. of grains spike^{-1}		Test weight (g)	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
Land configuration								
75 cm bed, 2 rows	12.3	12.5	20.8	22.1	54.7	56.8	43.46	43.80
75 cm bed, 3 rows	11.3	11.5	19.3	20.3	51.0	53.0	41.40	41.67
90 cm bed, 2 rows	12.1	12.3	24.2	25.2	55.9	58.6	44.15	44.67
90 cm bed, 3 rows	11.0	11.3	23.9	25.2	55.4	57.8	44.01	44.41
90 cm bed, 4 rows	11.0	11.2	21.3	22.3	52.8	54.4	43.51	43.87
Flat planting	10.1	10.3	18.8	19.8	50.0	52.3	42.74	42.95
SEm(±)	*0.06*	*0.07*	*0.32*	*0.20*	*0.35*	*0.45*	*0.14*	*0.15*
C.D. (P=0.05)	*0.18*	*0.22*	*1.00*	*0.63*	*1.11*	*1.41*	*0.45*	*0.46*
Irrigation schedules								
4 cm irrigation at IW/CPE 0.8	12.1	12.2	22.7	24.1	55.0	57.7	44.17	44.58
5 cm irrigation at IW/CPE 1.0	11.4	11.9	21.6	22.6	53.0	55.4	43.72	43.23
6 cm irrigation at IW/CPE 1.2	10.5	10.4	19.4	20.4	46.8	47.9	40.75	40.87
SEm(±)	*0.05*	*0.05*	*0.12*	*0.11*	*0.20*	*0.20*	*0.10*	*0.12*
C.D. (P=0.05)	*0.14*	*0.14*	*0.36*	*0.32*	*0.57*	*0.58*	*0.29*	*0.34*
Interaction I × B	Sig	Sig	Sig	Sig	Sig	Sig	NS	NS

Yield

The grain (46.52, 47.63 q ha^{-1}), straw (60.57, 61.55 q ha^{-1}), biological 107, 109.40 q ha^{-1}) yields and harvest index (43.39 and 43.49) significantly higher (Table 2) were recorded with $B_{90\text{-}4}$ land configuration the as compared to all other treatments during experimentation. The grain yield increased 11.00 and 12.02 %, straw yield 7.0 and 7.3% with $B_{90\text{-}4}$land configuration over flat planting during first and second year, respectively. Treatments $B_{75\text{-}4}$ (B_2) and flat planting (B_6) were at par with each other during both the year of study. However, $B_{90\text{-}2}$ (B_3) was recorded the lowest grain yield during both the year of study. Higher grain yield with bed planting of wheat has been also reported by (Bhahma*et al.* 2007; Kumar 2010; Thind*et al.* 2010).

The results have clearly shown that the grain yield in land configurations $B_{75–2}$, $B_{75–3}$, $B_{90–2}$ and $B_{90–3}$ was lower than that in flat planting due to low plant density, but the yield was higher in $B_{90–4}$ (B_5) than flat planting. The irrigation schedules having good tillering and higher rates of photosynthesis, had high biomass production and therefore was more suited for furrow irrigated raised bed planting system than flat planting.

Among the irrigation schedules IW/CPE 0.8 (I_1) and IW/CPE 1.0 (I_2) produced higher number of spikes

and biological yield than IW/CPE 1.2 (I_3). The significantly higher grain, straw, biological yields and harvest index was obtained in IW/CPE 0.8 (I_1) irrigation schedules and increased the grain yield 17.27 and 17.02 % over IW/CPE1.2 (I_3).

Interaction effects between irrigation schedules and land configuration in relation to grain yield, straw yield and biological yield were significant (Table 2). The magnitude of increase in spike length due to improvement in moisture supply by irrigation with furrow irrigated raised beds was observed under IW/CPE 0.8 (I_1) irrigation schedules with B_{90-2} land configuration as compared to other treatments combination during 2014-15 and 2015-16, respectively.

Table 2. Effect of land configuration and irrigation schedules on grain, straw, biological yield and harvest index

Treatment	Grain yield (q ha^{-1})		Straw yield (q ha^{-1})		Biological yield (q ha^{-1})		Harvest index (%)	
	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16	2014-15	2015-16
75 cm bed, 2 rows	39.33	40.18	55.33	56.42	39.33	40.18	55.33	56.42
75 cm bed, 3 rows	40.28	41.39	56.47	57.20	40.28	41.39	56.47	57.20
90 cm bed, 2 rows	37.80	38.57	54.63	55.36	37.80	38.57	54.63	55.36
90 cm bed, 3 rows	43.06	44.12	57.79	58.33	43.06	44.12	57.79	58.33
90 cm bed, 4 rows	46.52	47.63	60.57	61.55	46.52	47.63	60.57	61.55
Flat planting	41.92	42.52	56.59	57.35	41.92	42.52	56.59	57.35
SEm(±)	*0.57*	*0.54*	*0.74*	*0.80*	*0.57*	*0.54*	*0.74*	*0.80*
C.D. (P=0.05)	*1.79*	*1.70*	*2.34*	*2.50*	*1.79*	*1.70*	*2.34*	*2.50*
4 cm irrigation at IW/CPE 0.8	44.01	44.88	59.00	59.94	44.01	44.88	59.00	59.94
5 cm irrigation at IW/CPE 1.0	42.92	43.97	58.56	59.47	42.92	43.97	58.56	59.47
6 cm irrigation at IW/CPE 1.2	37.53	38.35	53.14	53.82	37.53	38.35	53.14	53.82
SEm(±)	*0.18*	*0.18*	*0.28*	*0.29*	*0.18*	*0.18*	*0.28*	*0.29*
C.D. (P=0.05)	*0.51*	*0.53*	*0.83*	0.86	*0.51*	*0.53*	*0.83*	0.86
Interaction I × B	Sig	Sig	Sig	Sig	Sig	Sig	NS	NS

Consumptive use

The consumptive use of water (23.0 and 23.8 cm) was more under flat method **(Table 3)** followed by the B_{75-2}, B_{75-3}, B_{90-2}, B_{90-3} and lowest value of consumptive use was recorded under B_{90-4}land configuration during both the year of study. The consumptive use of water directly related with moisture depletion and it washigher under flat method and lowest under bed B_{90-4}land configuration. During 2015-16 total consumptive use of water was more than 2014-15.

Treatment	Consumptive Use (cm)		Water use efficiency (kg m^{-3})	
	2014-15	2015-16	2014-15	2015-16
Land configuration				
75 cm bed, 2 rows	22.2	22.8	1.77	1.76
75 cm bed, 3 rows	21.6	22.1	1.86	1.87
90 cm bed, 2 rows	19.3	20.0	1.96	1.93
90 cm bed, 3 rows	19.2	19.7	2.24	2.24
90 cm bed, 4 rows	18.4	19.0	2.53	2.51
Flat planting	23.0	23.6	1.82	1.80
Irrigation schedules				
4 cm irrigation at IW/CPE 0.8	23.2	23.8	1.90	1.88
5 cm irrigation at IW/CPE 1.0	21.5	22.1	1.99	1.99
6 cm irrigation at IW/CPE 1.2	17.2	17.6	2.19	2.18

The consumptive use of water directly related with moisture depletion and it was higher under flat method and lowest under B_{90-4} (B_5) land configuration. So, consumptive use of water was also in the order of moisture depletion. During first year total consumptive use of water was more than the

second year mainly due to the differences in weather conditions, such as hot and dry wind and lesser number of rainy day.

Table 3. Effect of land configuration and irrigation schedules on consumptive use and water-use efficiency

Consumptive use by the crop includes total soil moisture depletion (cm) and soil moisture contributes Irrigation schedule of 4 cm irrigation IW: CPE 0.8. In contrast, the lowest consumptive use of water (17.2 and 17.6 cm) was under the irrigation schedule of IW: CPE1.2 due to combination of higher surface evaporation and moretranspiration so that moisture stresses condition occurs (Ahamad 2002; Maurya and singh 2008).

The consumptive use of water showed an increasing trend with increase in irrigation water during both the years. The highest consumptive use was recorded with irrigation schedule of IW: CPE 0.8 (I_1). This was mainly due to fact that the greater loss of applied water through evapotranspiration because of more availability of water resulted into better foliage and ultimately better plant growth. As a result of this was greater absorption of moisture by crop favored by highest water use at wettest regime. In contrast, the lowest consumptive use of water (17.6 and 17.2 cm) was under the irrigation schedule of IW: CPE 1.2 (I_3) due to combination of lower surface evaporation and reduced transpiration under less moisture availability.

Water-use efficiency

It is evident from the data (Table 3)that highest wateruseefficiency was recorded (2.53 and 2.51kg m^{-3}) under B_{90-4} (B_5) land configuration over flat planting method (B_6) during both the year of study. Treatment B_{90-4} (B_5) increased 39.01and 39.44%over flatmethodduring 2014-15 and 2015-16, respectively. This might be due to higher grain yield obtained under B_{90-4}(B_5) land configuration with lesser amount of water used. Declined water-use efficiency (WUE) under flatmethod with IW: CPE 0.8 (I_1) might be due to fact that grain yield did not increaseproportionately to that of consumptive use under this treatment.

An examination of data (Table 3) clearly indicates thatwater-use efficiency decreased with increase in levels ofirrigation during both the years. Maximum value of WUE, 2.19 and 2.18kgm^{-3}, were noted inIW: CPE 1.2 (I_3) during first and second yearsrespectively. It increased 15.2 and 16.0%overIW: CPE 0.8 (I_1) during first and second year, respectively. However minimum water-use efficiency wasunder IW: CPE 0.8 (I_1) during both the years. Decrease in WUE with IW: CPE 0.8 (I_1) basedon the fact that the proportionate increase in grain yield wasless than increase in the consumptive use of water.

Water productivity

The maximumwater productivity was registered (2.45and 2.98 kg m^{-3}) under B_{90-4} land configuration, followed by B_{90-3}> flat, B_{75-3}>B_{75-2}>B_{90-2}, treatments during both the years.

Higher water productivity (2.21kg m^{-3}) was affected by irrigation schedule of IW: CPE 1.2 (I_3) during 2014-15,but during 2015-16 higher water productivity(2.93 kg m^{-3}) was observed under IW/CPE 1.0 (I_2).Increase in water productivity (Table 4) with IW: CPE 1.2 (I_3) based on the fact that the proportionate increase in grain yield with lesser number of irrigations during experimentation(Kumar 2010; Singh et al., 2015).

In general, water productivity affectedby irrigation schedules the higher water productivity observed with IW: CPE 0.8 (I_1) during both the year of study. However minimum water productivity was observed in IW: CPE 1.2 (I_3).Decrease in water productivity with IW: CPE 1.2 (I_3),

Table 4. Effect of land configuration and irrigation schedules on water productivity Total water used by the crop includes applied irrigation and effective rainfall

Treatment	Total water applied (cm)		Water Productivity (kg/m^{-3})	
	2014-15	2015-16	2014-15	2015-16
Land configuration				
75 cm bed, 2 rows	19	16	2.07	2.51
75 cm bed, 3 rows	19	16	2.12	2.59
90 cm bed, 2 rows	19	16	1.99	2.41
90 cm bed, 3 rows	19	16	2.27	2.76
90 cm bed, 4 rows	19	16	2.45	2.98
Flat planting	19	16	2.21	2.66
Irrigation scheduling				
4 cm irrigation at IW/CPE 0.8	21	16	2.10	2.81
5 cm irrigation at IW/CPE 1.0	20	15	2.15	2.93
6 cm irrigation at IW/CPE 1.2	17	18	2.21	2.13

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RESEARCH ARTICLE

BIO-EFFICACY OF AZOXYSTROBIN 11% + TEBUCONAZOLE 18.3% SC ON ONION IN ANDHRA PRADESH

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Abstract: Field trials conducted against Azoxystrobin 11% + Tebuconazole 18.3% SC on Onion in Andhra Pradesh. Experimental findings with the data pertaining to efficacy of different fungicidal formulation on the purple blotch incidence showed that all the treatments were significantly superior over control in reducing the disease severity. Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 & 1000 ml/ha is superior and lowest disease incidence was recorded (17.15 & 18.05 respectively) and proved to be the best. Highest yield was obtained in treatment sprayed with the Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha and it was on par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750ml/ha with the highest cost benefit ratio of 1:2.16.

Keywords: Bio-efficacy, Azoxystrobin, Tebuconazole, Purple blotch

INTRODUCTION

Onion *(Allium cepa* L.) is a high value spice cum bulbous vegetable crop cultivated in almost all parts of the country. In India, onion occupies an area of 0.52 million hectare with the production of 6.50 million tonnes. Even though India ranks first in area under onions in the world and second in production but its productivity is low (12.5 t/ha) as compared to worlds productivity (Anon., 2004), (Kappa Kondal, 2014).

Onion is cultivated throughout the year in Kurnool district, which is one of the largest producing onion district. The area is more in Gonegandla, Kodumuru, C. Belagal, Veldurthi, Bethamcherla, Orvakallu and Nandikotkuru mandals. The most important varieties are Bellary red, Agrifound light red, N-53 and Orient hybrid. The major constraints in onion production are spurious seed, uneven bulb development, price fluctuations and diseases. Among several factors, diseases are one of the most important factors associated with low productivity in onion. Purple leaf blotch caused by *Alternaria porri* is one among the serious fungal diseases that affect onion, causing heavy yield loss ranging from 2.5 to 87.8 per cent during *kharif* season (Srivastava *et al*., 1994).

MATERIAL AND METHOD

A field experiment was conducted at Horticultural Research Station, Mahanandi, Kurnool, (A. P.) to study the efficacy of Azoxystrobin 11% + Tebuconazole 18.3% SC in Agrifound dark red variety of onion with 30x15cm spacing and 5x5 M plot size during kharif 2013 and 2014. The experiment was laid out in Randomized Block Design (RBD) and the crop was raised by standard agronomic practices. The test fungicide, Azoxystrobin 11% + Tebuconazole 18.3% SC was evaluated at 3 doses 750, 1000 and 1250 ml/ha against purple blotch disease.

The crop was raised as per the recommended package of practices, except plant protection measures. The first treatment spray was done soon after the onset of the disease and subsequent two sprays were taken up at an interval of 15 days.

Rating scale for assessment of Purple blotch disease

Observations were recorded at first appearance of the disease symptoms on leaves till the harvest at weekly intervals. The percent disease intensity was recorded by using 0-5 scale for onion purple leaf blotch (Sharma, 1986).

The details of 0-5 scale (Sharma, 1986) in onion purple blotch

Grade	Description of the symptoms
0	No disease symptom
1	A few spots towards tip covering 10 percent leaf area.
2	Several purplish brown patches covering up to 20 percent of leaf area
3	Several patches with paler outer zone covering up to 40 percent leaf area.
4	Leaf streaks covering up to 75 percent leaf area or breaking of the leaves from center
5	Complete drying of the leaves or breaking of leaves from center.

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The percent disease index of purple leaf blotch in onion was calculated using the following formula:

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of the individual diseases grade x 100}}{\text{Number of leaves observed x Maximum Disease grade}}$$

Table 1. Evaluation of Azoxystrobin 11% + Tebuconazole 18.3% SC against Purple blotch in Onion in first season

Sl. No	Treatment	Formulation ml/ha	*Purple blotch	Onion Yield (t/ha)
T_1	Azoxystrobin 11% + Tebuconazole 18.3% SC	500	20.14 (26.64)	18.46
T_2	Azoxystrobin 11% + Tebuconazole 18.3% SC	750	17.26 (24.51)	20.24
T_3	Azoxystrobin 11% + Tebuconazole 18.3% SC	1000	16.54 (23.97)	21.25
T_4	Azoxystrobin 23% SC	500	22.05 (27.97)	19.00
T_5	Tebuconazole 25.9% EC	750	23.24 (28.79)	18.05
T_6	Difenconazole 25% EC	500	25.28 (30.13)	17.12
T_7	Control	-	29.55 (32.90)	13.80
CD 5%			1.88	1.15

*Figures in parenthesis are angular transformed value

Table 2. Evaluation of Azoxystrobin 11% + Tebuconazole 18.3% SC against Purple blotch in Onion in second season

Sl. No	Treatment	Formulation ml/ha	*Purple blotch	Onion Yield (t/ha)
T_1	Azoxystrobin 11% + Tebuconazole 18.3% SC	500	21.85 (27.83)	18.22
T_2	Azoxystrobin 11% + Tebuconazole 18.3% SC	750	18.05 (25.14)	23.06
T_3	Azoxystrobin 11% + Tebuconazole 18.3% SC	1000	17.15 (24.43)	23.84
T_4	Azoxystrobin 23% SC	500	22.84 (28.52)	19.80
T_5	Tebuconazole 25.9% EC	750	23.66 (29.06)	18.15
T_6	Difenconazole 25% EC	500	24.58 (29.67)	17.06
T_7	Control	-	30.25 (33.34)	14.54
CD 5%			1.50	1.21

*Figures in parenthesis are angular transformed value

RESULT

In first season, the data pertaining to efficacy of different fungicidal formulation on the purple blotch incidence showed that all the treatments were significantly superior over control in reducing the disease severity. Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 & 1000 ml/ha is superior and lowest disease incidence was recorded (17.26 & 16.54 respectively) and proved to be the best (Table 1). Highest yield was obtained in treatment sprayed with the Azoxystrobin 11% + Tebuconazole 18.3% SC @ 1000 ml/ha and it was on par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750ml/ha with the highest cost benefit ratio of 1:2.11 In second season, recorded the same results, that all the treatments were significantly superior over control in reducing the disease severity. Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750 & 1000 ml/ha is superior and lowest disease incidence was recorded (17.15 & 18.05 respectively) and proved to be the best (Table 2). Highest yield was obtained in treatment sprayed with the Azoxystrobin 11% + Tebuconazole 18.3% SC @

1000 ml/ha and it was on par with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750ml/ha

The cost benefit ratio calculated for different fungicides revealed the highest C:B ratio was found in with Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750ml/ha and 1000 ml/ha (1:2.11 and 1:1.01) respectively were found to be superior over rest of the treatments (Table No.3).

Table 3. Economics of using Azoxystrobin 11% + Tebuconazole 18.3% SC against Purple blotch in Onion

Sl. No	Treatment	Formulation ml/ha	Yield (t/ha)	Overall income (Rs./ha)	Economic Benefit over control
T_1	Azoxystrobin 11% +Tebuconazole 18.3% SC	500	18.46	97800	1:1.47
T_2	Azoxystrobin 11%+Tebuconazole 18.3% SC	750	20.24	106250	1:2.11
T_3	Azoxystrobin 11% +Tebuconazole 18.3% SC	1000	21.25	100700	1:2.01
T_4	Azoxystrobin 23% SC	750	19.00	95000	1:1.37
T_5	Tebuconazole 25.9% EC	400	18.05	85750	1:1.15
T_6	Difenconazole 25% EC	500	17.12	91100	1:1.26
T_7	Control	-	13.80	66000	-

DISCUSSION

The results were in accordance with the following reports. .Chethan *et al* (2013) revealed that mancozeb was most effective in reducing the disease intensity by 44.43% and increasing the yield by 43.57%. *T.harzianum* & garlic extract were the next best effective treatments to reduce the disease intensity by 32.05 and 27.19% and increased the yield by 39.63 and 34.94%. The incremental cost benefit ratio was recorded highest in mancozeb (1:14.60) followed by *T. harzianum* (1:10.05). in this study showed that mancozeb was the only fungicide found to be most effective than bioagents and botanicals. However all other botanicals and bioagents were at par with fungicides in reducing the disease intensity and increasing the yield? Thus the effective bioagents and botanicals could be employed individually or by integration with fungicides for the efficient management of purple blotch disease to ensure bioefficacy of fungicides against *Alternaria porri* (Ellis) Cif., causing purple blotch of onion (*Allium cepa* L.). Chethan *et al* (2011) studied on different fungicides and these findings were accordance with this new result.

Rao *et al.* (2015) also proved that Purple blotch disease caused by *Alternaria porri* is a major production constraint in onion, causing severe crop loss ranging between 30 and 100%. In this study, evaluated fungicides including new molecules for the management of purple blotch disease of onion. 10 nonsystemic fungicides,13 systemic fungicides and 6 combination products at different concentrations were evaluated against *A. porri* under *in vitro* condition. Among them a nonsystemic fungicide, Mancozeb 70% WP @ 2500 ppm and a combination product, Cymoxanil 8%+Mancozeb 64% @ 2500 ppm were best by completely inhibiting mycelial growth and conidial germination of *A. porri*.

Efath Shahnaz (2013) studied that foliar blight is an important disease of onion, proving a major bottleneck in its production. Six pathogens were found associated with the disease, *viz., Alternaria alternata*, *A. porri*, *A. tenuissima*, *Stemphylium vesicarium*, *Colletotrichum circinans* and *Cladosporium alliicepae*. Integrated disease management of the crop was attempted using chemicals (mancozeb at 0.25% and hexaconazole at 0.06%), biocontrol agents, *Trichoderma viride* (Tv1) and *Trichoderma harzianum* (Th1), each at 1×109 spores/ml and phytoextracts (*Cannabis indica* and *Curcuma longa*, each at 10%). Mancozeb at 0.25 per cent proved most effective in managing foliar blight of onion but was at par with hexaconazole at 0.06 per cent. Among biocontrol agents used, application of *T. harzianum* (Th1) resulted in lower disease intensity as compared to *T. viride* (Tv1), though both were statistically at par with each other, but were significantly superior over the control. The phytoextracts, *C. indica* and *C. longa* were ineffective in the disease management.

Sobhy II Abdel-Hafez *et a*l., investigated onion purple leaf blotch was controlled by plant extracts like *Azadirachta indica* under invitro conditions.

Rahman *et al.* (1989) proved maximum disease reduction was highest with Rovral, Dithane M-45 in controlling purple leaf blotch of onion.

Savitha (2015), significant effect was found with the seed treatment using Pseudomonas fluorescens (5 g/kg) followed by two sprays of difenconazole (0.1%) interspersed with P. fluorescens (0.5%) spray with a per cent disease index of 38.67 and bulb yield of 19.41 t/ha, which was significantly superior over other treatments and control. Whereas, the seed treatment with P. fluorescens followed by two sprays of iprodione + carbendazim (Quintal) (0.2%) interspersed with P. fluorescens (0.5%) spray was on par with standard check (two sprays of difenconazole at 0.1%) with the PDI of 42.00 and 43.33 and bulb yield of 16.64 and 15.68 ton/ha respectively.

CONCLUSION

It is evident from the present investigation during two seasons Kharif-2013 and Kharif-2014, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 750ml/ha showed with the highest cost benefit ratio of 1:2.11 and lowest purple leaf blotch disease incidence 17.26.(PDI)

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RESEARCH ARTICLE

ASSESSMENT OF HONEY DEW EXCRETION BY NON -TARGET BPH, *NILAPARVATA LUGENS* STAL.ONDIFFERENT IR-64 *BT* RICE EVENTS

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Abstract: The experiment was undertaken at greenhouse of Entomology and Department of Plant molecular biology &biotechnology, CoA, Raipur during 2014 and 2015.Area marked due to honey dew excretion by BPH under different IR64 Bt rice events ranged from 15.52 to 24.85 mm^2. The maximum marked area (24.85 mm^2) was observed in IR-64-C followed by TN-1-C (23.58 mm^2) with minimum in Ptb-33-C (15.52 mm^2) during 2014. Whereas during 2015, new starved female was released and new filter paper was kept inside the funnel to receive the honey dew in all the rice events were ranged from 11.72 to 20.43 mm^2. The maximum marked area (20.43 mm^2) was observed in IR-64-4 followed by IR-64-1 and TN-1-C (23.58 mm^2), respectively and minimum in Ptb-33-C (11.72 mm^2). On the basis of two years, pooled mean of honey dew area marked under different rice events was ranged 13.62 to 21.43 mm^2. The highest honey dew excreted on IR64 Bt events was noticed (21.43 mm^2) in IR-64-4 followed by TN-1-C (20.84 mm^2) and minimum in Ptb-33-C (13.62 mm^2) within 24hrs. releasing of BPH. The descending order of honey dew excretion by starved female on Bt events was as IR-64-4> TN-1-C > IR-64-C> IR-64-1> IR-64-2> Ptb-33-C. The area of honey dew excretion by female on *Bt* rice and on non-transgenic control rice plants did not differ significantly.

Keywords*:* Bt protein, Non-target insect BPH, Honey dew excretion

INTRODUCTION

Rice is the most remunerative crop stands first among all food grain and is staple food for more than half of world's population. Insect pest are one of the major constraints of high tech agriculture and pesticides use is necessary .The transgenic plants expressing insecticidal properties are becoming environmentally safe alternatives to chemical pesticides. Genetically modified crop containing crystal protein from the bacterium *Bacillus thuringiensis* (Bt) was grown on 26.3 million ha worldwide in the year 2005 (James, 2005). Bt rice has the potential to eliminate yield losses caused by lepidopteron pests up to 2%-10% of Asia's annual rice yield of 523 million tons (High *et al.,* 2004). Genetically modified crops had provided economic benefits to growers and also offer a promising alternative to chemical insecticides for control of lepidopteran pests in rice (Zhu, 2001; High *et al.,* 2004). Zhou *et al*., (2004) had detected the impact of Bt rice on non -target *Nilaparvata lugens* and he did not find any difference in feeding and oviposition behavior. In recent years, rice stem borers had developed resistance to some most commonly used insecticides in China and other rice growing countries. The transgenic rice on target lepidopteran pests is a important tool both for pest management and insecticide resistance management. The Bt rice has effectively controlled the three species of stem borers (*C. suppressalis, S. incertulas, S. inferens*) and leaf folder (*C. medinalis*) as reported by Tu*et al.* (2000).The rice field has highly diverse and interlinked insect pest species of herbivores, predators, and parasitoids. These are an essential component of biological control and are one of the fundamentals of insect management strategies in rice (Schoenly *et al* 1998). This importance was previously exemplified by the outbreaks of brown plant hopper, *Nilaparvata lugens* (Homoptera:Delphacidae) that resulted from the excessive use of insecticides in early 1997 (Gallagher *et al*1994). For biological control in managing the balance of insect pest population in rice fields, it is essential to assess the effect of novel insecticides such as *Bt* on non-target insects and their predators/parsitoids. Mirid bug, *Cyrtorrhinus lividipennis* (Hemiptera: Miridae) survives predaciously by feeding on *N. lugens* larvae and nymphs in the rice ecosystem. Insight into the potential effect of the deployment of transgenic *Bt*rice on the population dynamics of other non-target insects and their predatory organisms can be gained by evaluating the effects of Bt toxins on life-history parameters of brown planthopper and mirid bug feeding on *Bt* rice and control non-*Bt* rice.

This study was undertaken to assess the effect of Bt toxins on life-history parameters of brown plant hopper, a non-target insect of rice to understand the secondary exposure of Bt toxins. *N. lugens* is a major pest of rice and an important constituent of the

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population structure of the rice growing area of Asian countries.

MATERIAL AND METHOD

Experimental details

The experimentalwas undertaken at glasshouse of Entomologyand Department of Plant molecular biology & biotechnology, College of Agriculture, Raipur during 2014 and 2015.

Mass culture of BPH

The macropterous females of *N. lugens*50 to 100 per field were collected from Entomological rice fields of IGKV,Raipur. These females were pooled and allowed to oviposit on caged rice plants of Taichung Native-1 (TN-1), a rice genotype that does not contain any genes with resistance against these pest species. From the resulting progenies,colonies weremaintained on susceptible rice variety TN-1.The culture of BPH is being maintained throughout the experimental period in the air cooled glass house in the Department of Entomology at 30°C ± 5°C on potted TN1 variety of rice. BPH were reared on 40 to 45 days old potted TN1 plants inside a rearing cage of 75 x 75 x 75 cm size, consisting of wooden frame with small window on front side and fine wire mesh on top and other sides. Cages were mounted on cemented platform having water level of 7.5 cm. Potted TN1 plants were placed inside the rearing cages for egg laying along with at least 60 pairs of BPH per pot. After 2-3 days the females starts egg laying inside the leaf sheath at the basal portion of paddy plants. After the emergence of nymphs from plants BPH pairs were transferred to another TN1 pots with the help of aspirator for egg laying.The colonies were grown on an artificial diet before releasing them onto transgenic *Bt* rice plants.The BPH population was taken from mass culture maintained in the glasshouse. Standard evaluation technique developed by IRRI was adopted to evaluate different Bt/non Bt lines. Observations were recorded on the honey dew excretion for all the rice events.

Collection and quantification of honeydew of *N. lugens*

The parafilm sachet method as described by Pathak *et al.* (1982) was used to collect honeydew from the female adults of *N. lugens* fed on transgenic *Bt*rice and their counterpart control rice lines. Three sachets were attached to the stems of each plant. Plants were grown in the transgenic greenhouse arranged in a complete block design (CBD) at 25-30°C under a natural photoperiod of approximately L12:D12. Three fifth instar nymphs were randomly selected from *N. lugens* colonies maintained on caged TN-1 rice plants and starved for 2 hrs were then placed singly into inverted plastic cups enclosing a plant stem and allowed to feed for 24 hrs. After 24hrs, the filter papers were collected and the area of honeydew spots on the filter paper was determined by placing the transparent sheets with 1 mm grids on top of the filter paper. Blue and white spots, produced by alkaline and acidic honeydew deposition, respectively, were determined separately. The honeydew excreted was collected with the help of a micropipette and placed into 1.5-ml micro centrifuge tubes. The honey from each plant was pooled and stored at -20°C/-80°C. There were three replicates for each transgenic line and three for their control plants.The presence of Cry protein in the honeydew secretions was analyzed using the CryIAb/CryIAc ELISA kit (Envirologix, USA) at 450 nm as per the manufacturer's instructions Reading for each replication was recorded separately. Honeydew production on rice plants at the maximum tillering stage was quantified using the bromocresol green technique (Pathak and Heinrich 1982).

Table 1. Honeydew excretion by BPH on differenttransgenic rice (including control) lines during 2014 and 2015

Transgenic rice	Honeydew excretion by BPH within 24h (mm^2)		
	2014	2015	Pooled mean
IR-64-1	18.98 (4.41)	18.10 (4.31)	18.54
IR-64 -2	19.10 (4.43)	17.89 (4.29)	18.49
IR-64-3	20.18 (4.55)	14.93 (4.93)	17.56
IR-64-4	22.42 (4.79)	20.43 (4.58)	21.43

IR-64-C	24.85 (5.03)	14.74 (4.90)	19.80
TN1-C	23.58 (4.91)	18.10 (4.31)	20.84
Ptb33-C	15.52 (4.00)	11.72 (3.50)	13.62
SEM	**4.32**	**2.90**	
CD at 5%	**7.57**	**5.09**	
CV (%)	**36.23**	**30.37**	

*The values in parenthesis are square root transformed values

* Three replications for each treatment

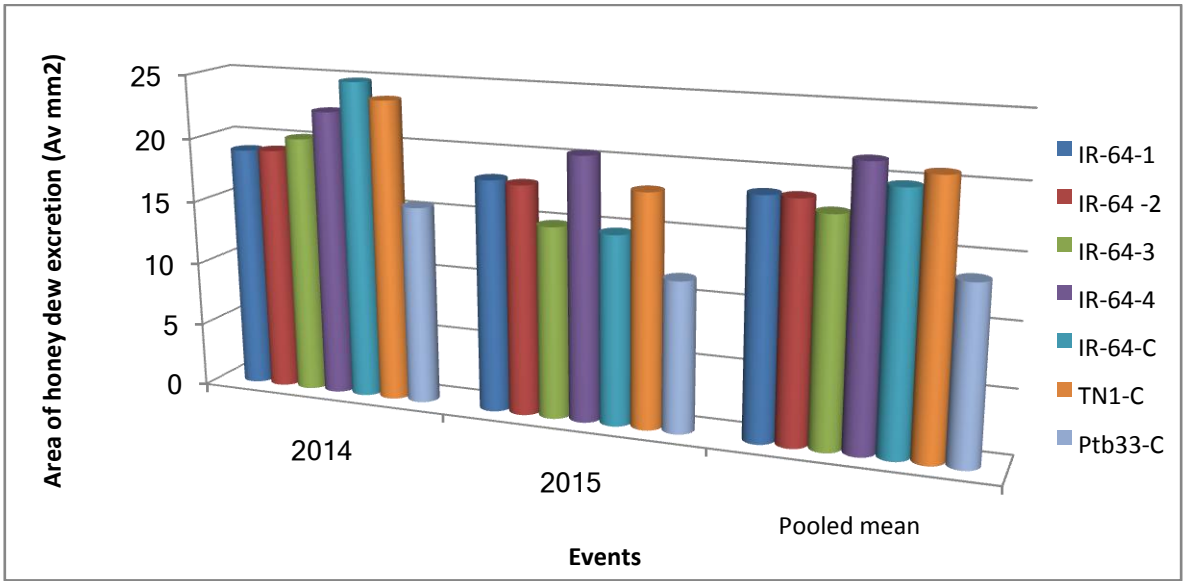

Fig.1. Honeydew excretion by BPH on differenttransgenic rice (including control) lines during 2014 and 2015

RESULT AND DISCUSSION

Area marked due to honey dew excretionby BPH under different IR64 Bt and non-Bt events ranged from 15.52 to 24.85 mm^2. The maximum marked area (24.85 mm^2) was observed in IR-64-C followed by TN-1-C (23.58 mm^2) with minimum in Ptb-33-C (15.52 mm^2) during 2014. Whereas during 2015, new starved female was released and new filter paper was kept inside the funnel to receive the honey dew in all the treatment and replications were ranged from 11.72 to 20.43 mm^2. The maximum marked area (20.43 mm^2) was observed in IR-64-4 followed by IR-64-1 and TN-1-C (23.58 mm^2), respectively and IR-64-4 (22.42 mm^2)and minimum in Ptb-33-C (11.72mm^2). On the basis of two years, pooled mean of honey dew area marked under different treatments was ranged 13.62 to 21.43 mm^2. Maximum honey dew excreted onIR64 Bt and non-Bt events was noticed (21.43 mm^2) in IR-64-4 followed by TN-1-C (20.84 mm^2) and minimum in Ptb-33-C (13.62 mm^2) within 24hrs releasing of BPH (table-1 & Fig.-1).

The honeydew excreted by females of *N. lugens* was quantified by measuring the area of white and blue spots on bromocresol green-treated filter paper. White spots, indicating the deposition of acidic honeydew from xylem feeding, were significantly fewer in the transgenic *Bt* rice lines than in the corresponding control plants. whereas, the area of blue spots indicating the feeding from the phloem region was significantly higher in transgenics than in the control rice plants. Our results clearly indicate that BPH feeding on *Bt* rice has shown a preferred feeding behavior from phloem tissues. The results observed with the honeydew excreted by *N. lugens* females feeding on *Bt* rice observed are similar to those in an earlier study by Bernal *et al* (2002) where toxic protein was detected in honeydew and phloem tissues of *Bt* rice plants.

On the basis of honey dew area marked under different treatments at 48hrsreleasing of BPH, it may be stated that starved female feeding on IR-64-4Bt plant, honey dew excretion increased gradually and it was higher to other Bt and non- Bt control whereas, in case ofPtb-33-C treated plant, the insect start

feeding subsequent but thequantum of feeding was low as compared to other Bt events within 24hrs.The descending order of honey dew excretion bystarved female on Bt events wasas IR-64-4> TN-1-C> IR-64-C> IR-64-1> IR-64-2>Ptb-33-C. Are of honey dew excretion by female on *Bt* rice and on non-transgenic control rice plants did not differ significantly.

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RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF SOME MEDICINAL PLANT EXTRACTS AGAINST HUMAN PATHOGENIC FUNGUS *ASPERGILLUS NIGER*

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Abstract: The present investigation was carried out to observe the antifungal activity of *Alstonia scholaris Argemone maxicana, Datura alba, Solanum nigrum and Solanum xanthocarpum.* For this purpose effect of different alcoholic extract concentration was observed on growth performances of *Aspergillus niger* on 5th and 7th day. Our result shows that alcoholic extract concentrations inhibit radial growth of this fungus. Results also indicate that inhibition of fungal growth increase with the increase in the concentration of alcoholic extracts.

Keywords: Antifungal activity, Alcoholic extract, *Aspergillus niger*, Medicinal plants

INTRODUCTION

Herbal medicines used against various fungal diseases. Antifungal activity of natural plant extract and pure compound can be detected by inhibition of various microflora like yeast, fungi by samples that are placed with them. About 100,000 species of fungus are present in the environment and more than 100 of them are pathogenic in human (Keeler 1991). Many of the Pharmaceuticals like opium, aspirin, digitalis, quinine etc have a long history of usage as herbal remedies. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (Cragg et al., 1997 and Shu, 1998). Plants and their extracts have been used all over the world in folk medicines and the use of extracts has been supported by the isolation of antifungals from the plants. Many plants produce secondary metabolites. These metabolites may serve as potent antimicrobial agents and thus may be useful for human beings. It has been estimated by the World Health Organization (WHO) that 80 percent of the population of some Asian and African countries presently use herbal medicine for some aspect of primary health care (Akerele, 1993). Lupeol and Epicatechin have been identifies in the methanol extract of *Alstonia scholaris.* This extract has shown antioxidant and anticancer effect. It also showed significant antimicrobial effect against *Staphylococcus aureus* and gram negative organisms like *Escherichia coli, Proteus vulgaris, Pseudomonas auriginosa* and *Candida albicans* (Thara and Zuhra, 2013). Traditionally herbal medicines provide an interesting, largely unexplored source of potential new drugs (Udgirkar *et al*., 2012). Antifungal activity of eight medicinal plants extract (*Aloe vera, Ocimum sanctum, Cenetella asiatica, Piper betle, Calotropis gigantea, Vitex negundo, Ocimum basilicum* and *Azadirachta indica*) was assayed by agar well diffusion method on plant pathogenic fungus (red rot disease causing agent) *Colletotrichum falcatum*. The result revealed that the extract of eight medicinal plants showed significant reduction in growth of *C. falcatum* (Prince and Prabakaran, 2011). Antony *et al*., 2012 have reported that butanolic extract of bark of the *Alstonia scholaris* have potent anti-tubercle effect and anti-*Mycobacterium tuberculosis* potential and it was concluded that it is a promise for future therapeutic interventions. Therefore present investigation has been aimed to evaluate the antifungal activity of alcoholic extracts of five medicinal plants against the pathogenic fungus viz. *Aspergillus niger.*

MATERIAL AND METHOD

Sample Collection

Samples for the following medicinal plants were collected from district Saharanpur & Shiwalik belt of Uttar Pradesh as well as from Garhwal hills of Uttarakhand, India.

1. *Alstonia scholaris*
2. *Argemone maxicana*
3. *Datura alba*
4. *Solanum nigrum*
5. *Solanum xanthocarpum*

The freeze-dried pathogenic fungi *Aspergillus niger* was obtained from Forest Pathology Division, Forest Research Institute, Dehradun. The cultures were maintained on Sabouraud Dextrose Agar (SDA) slants and kept refrigerated until used. The SDA plate cultures were inoculated from the slants and incubated at 25 ± 1°C for 7 days.

Plant Extract Preparation

For the preparation of various plant extracts 5 gm of fresh plant part was washed 2-3 times with distilled water and then treated with 0.1% $HgCl_2$ solution for sterilization. After surface sterilization plant samples were ground in mortar and pestle with 50% methanol. The homogenized liquid was filtered and centrifuged at 5000 rpm. The supernatants were used as test extract & make up into 20 ml using 50% methanol. Further, the extract was diluted into

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different concentrations, i.e. 10%, 25% and 50%. 20 ml of SDA (Sabouraud Dextroes Agar) culture medium with 5 ml of the above concentration of the extracts were poured in sterile petriplates and allowed to solidify. In the control same volume of distilled water (in place of experimental material) was mixed in appropriate amounts.

Fungal Inoculation

For antifungal activity mycelia discs of 5 mm diameter were cut from the periphery of 7 day old culture of the test organisms and were aseptically inoculated upside down on the surface of the SDA medium in plates. Inoculated petriplates were incubated at 25^0 C ± 1^0C and observation were recorded at 5^{th} and 7^{th} day.

After 5^{th} and 7^{th} day of incubation, observations were recorded on the basis of colony diameter (cm) on medium and percent inhibition of radial growth was calculated using following formula:

$$\% \text{ Growth Inhibition} = \frac{\text{Colony diameter in control} - \text{Colony diameter in treated sets}}{\text{Colony diameter in control}} \times 100$$

OBSERVATION AND RESULT

The present investigation was carried out to observe the antifungal activity of *Alstonia scholaris, Argemone maxicana, Datura alba, Solanum nigrum and Solanum xanthocarpum*. For this purpose effect of different alcoholic extracts concentrations with (10%, 25% and 50%) were observed on the growth performances of *Aspergillus niger* causing human skin diseases are given in Table 1.

Antifungal activity of Alstonia scholaris on Aspergillus niger

Table 1 shows that in 10%, 25% and 50% alcoholic root extracts of *Alstonia scholaris* the growth of *Aspergillus niger* was 75.0%, 62.5%, 50% control respectively in 5^{th} day old culture plate. Similarly, the growth is inhibited in presence of alcoholic shoot and seed extracts in culture medium. However, the inhibition of growth found more at higher concentrations. So in presence of 50% alcoholic shoot and seed extract the growth of *Aspergillus niger* is 43.3% and 48.3% of control respectively at 7^{th} day. Nearly similar pattern of growth inhibition found in various other concentrations at both days of studies.

Antifungal activity of Argemone maxicana on Aspergillus niger

Observation shows that alcoholic extracts of *Argemone maxicana* plant part is inhibitory to the growth of *Aspergillus niger*. Results have shown that higher concentration of alcoholic extract is inhibitorier as compared to the lower concentration of alcoholic extracts. Thus, 50% root extract causes 67% growth inhibition at 7^{th} day. Likewise, growth of *Aspergillus niger* on both day in various concentrations of seed extracts also inhibited. Thus, in 50% alcoholic seed extract at 5^{th} day and 7^{th} day the growth is 50.0% and 55.5% of control respectively.

Table 1 also shows that shoot extract concentrations are also inhibitory to the growth of *Aspergillus niger*. Thus, in 10%, 25%, and 50% alcoholic root concentration the fungal growth at 7^{th} day is 90.0%, 70.0% and 46.6% of control respectively.

Antifungal activity of Datura alba on Aspergillus niger

Result in table 1 shows that alcoholic plant part extracts of *Datura alba* are inhibitory to the growth of *Aspergillus niger*. Results have shown that inhibition of fungal growth increases with the increase in the concentrations of alcoholic extracts. Thus, 10% alcoholic seed extract causes 18.0% inhibition of *Aspergillus niger* growth at 7^{th} day, however, this inhibition in 50% alcoholic seed extract at 7^{th} day is ca. 52%. Root and shoot extracts of this plant also inhibits the growth of *Aspergillus niger* in culture medium. Thus, in 10%, 25%, and 50% alcoholic root extract the Fungal mycelial growth is 85.7%, 68.8% and 53.5% of control respectively at 7^{th} day. Likewise, in 10%, 25% and 50% shoot extract concentration the mycelial growth is 83.3%, 66.6% and 46.6% of the control respectively at 7^{th} day of growth.

Antifungal activity of Solanum nigrum on Aspergillus niger

Studies have shown that with the increase in concentration of alcoholic extract the inhibition of radial growth of Fungi also increases. Thus, in 10%, 25% and 50% alcoholic concentration of root extract the growth is 85.2%, 70.50% and 50.0% respectively of the control at 7^{th} day of radial growth.

Table 1 shows that like root extract growth of *Aspergillus niger* is also inhibited in the various concentrations of shoot and seed extracts. Result shows that 50% alcoholic seed extract is inhibitory by 42% at 7^{th} day of radial growth of *Aspergillus niger*.

Antifungal activity of Solanum xanthocarpum on Aspergillus niger

Result shows that *Solanum xanthocarpum* carries fungicidal property to control the growth of this fungi. Observation further shows that alcoholic extract of various plant parts of this plant retards the radial growth of this fungi. Thus, radial growth of this fungi on 7^{th} day in presence of 10%, 25% and 50% alcoholic root extract are 80.0%, 72.0% and 52.0% of the control respectively.

Result further shows that like root extract growth of this fungi also inhibited in the presence of shoot

extract and seed extract. Thus, in 50% alcoholic extract concentration of shoot and seed at 7th day the growth is 58.3% and 57.6% of the control respectively. Thus, the above studies shows that the growth of this fungi affected by the alcoholic extract of above medicinal plant.

Table 1. Antifungal activity of *Alstonia scholaris, Argemone maxicana, Datura alba, Solanum nigrum, Solanum xanthocarpum* on growth performance of *Aspergillus niger*

Days	*Alstonia scholaris*			*Argemone maxicana*			*Datura alba*			*Solanum nigrum*			*Solanum xanthocarpum*		
	Root	**Shoot**	**Seed**	**Root**	**Shoot**	**Seed**	**Root**	**Shoot**	**Seed**	**Root**	**Shoot**	**Seed**	**Root**	**Shoot**	**Seed**
Growth in Control 0% extract															
5th	2.4	2.6	2.8	2.1	1.6	1.8	2.6	2.3	2.4	2.6	2.9	2.7	1.6	1.7	1.6
7th	2.8	3.0	3.1	3.0	2.8	2.7	2.8	3.0	2.9	3.4	3.2	3.1	2.5	2.4	2.6
Growth in 10% alcoholic extract															
5th	1.8	2.4	2.6	1.8	1.5	1.4	1.6	1.8	1.7	2.0	2.0	1.8	1.3	1.4	1.3
7th	2.0	2.8	3.0	2.7	2.5	2.0	2.4	2.5	2.4	2.9	2.8	2.6	2.0	2.0	2.1
Growth in 25% alcoholic extract															
5th	1.5	1.6	1.8	1.2	1.1	1.0	1.3	1.4	1.2	1.6	1.4	1.5	1.0	1.1	1.0
7th	1.9	2.2	2.0	2.1	2.2	2.0	1.9	2.0	1.8	2.4	2.3	2.1	1.8	1.7	1.8
Growth in 50% alcoholic extract															
5th	1.2	1.3	1.6	1.0	0.8	0.9	1.0	1.2	1.2	1.4	1.2	1.3	0.8	0.9	0.9
7th	1.7	1.3	1.5	1.4	2.0	1.5	1.5	1.4	1.4	1.7	2.0	1.8	1.3	1.4	1.5

DISCUSSION AND CONCLUSION

Studies on herbal plant extracts showed that the various solvent extracts showed promising antimicrobial activity against fungal human pathogens. These extracts can be utilized for isolation and characterization of therapeutically active chemical constituents used in modern medicines. Alcoholic plant extract used here showed significant antifungal activity against *Aspergillus niger*. So this antifungal property provides a scientific basis for the use of these plants as suitable antifungal agent. This extract can be used against infection caused by *Aspergillus niger*. This study also encourages that these plant should be cultivated in large scale to increase the use of these plant in traditional medicine. Results with different alcoholic extract concentration of *Alstonia scholaris, Argemone maxicana, Datura alba, Solanum nigrum and Solanum xanthocarpum* on the radial growth of pathogenic fungus like *Aspergillus niger*, clearly shows that alcoholic extract concentration inhibits radial growth of opportunistic fungi. Result indicates that inhibition of fungal growth increase with the increase in the concentration of alcoholic extracts.

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RESEARCH ARTICLE

EFFICACY OF BIO-AGENTS AND ORGANIC AMENDMENTS AGAINST *SCLEROTIUM ROLFSII* CAUSING COLLAR ROT OF CHICKPEA

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Abstract: Chickpea is cultivated throughout the Chhattisgarh state and mostly grown in kanhar soil in Chhattisgarh plains. However, chickpea productivity is low due to susceptibility of the crop to different biotic and abiotic stresses. The collar rot disease of chickpea caused by *Sclerotium rolfsii,* which is soil borne and fast spreading fungus, causes considerable damage to the plant stand. The collar rots of chickpea caused by *S. rolfsii,* can cause considerable loss to plant stand when soil moisture is high and temperature is warm (nearly 30^0C) at sowing time. Drying of plants with foliage turned slightly yellow before death, scattered throughout the field is an indication of collar rot infection. The study of bio-agent and organic amendment application revealed that all the treatments significantly increased seed germination and reduced collar rot incidence. Seed treatment with bio-agent *Trichoderma* and Neem cake application in soil was found to be the most effective recording maximum seed germination and minimum mortality followed by *Trichoderma* with Mustard cake and *Trichoderma* with Karanj cake combination under natural condition.

Keywords: Collar rot of chickpea, *Sclerotium rolfsii, Trichoderma spp,* Bio-agents

INTRODUCTION

Chickpea is an important pulse crop grown all over the world; it occupies the premier position in terms of area as well as production. In India, chickpea is grown over 6.93 m ha with the production of 5.60 m tones. Chickpea contributes about 37 per cent of the total pulse production in the country. Chhattisgarh contributes a 0.26 m tone that is about 4.43 per cent of total chickpea production of India (Anonymous, 2008). *Sclerotium rolfsii* causing collar rot is an important soil borne and fast spreading fungal pathogen causes considerable damage to economically important crops like (chickpea, soybean, groundnut, beans, clover, peas and lentil). Under field conditions, the *S. rolfsii* has been reported to cause 30 to 60 per cent reduction in yield of chickpea (Prasad, 2005). The collar rots of chickpea caused by *S. rolfsii,* can cause considerable loss to plant stand when soil moisture is high and temperature is warm (nearly 30^0C) at sowing time. Drying of plants with foliage turned slightly yellow before death, scattered throughout the field is an indication of collar rot infection. The disease generally appear within two weeks of sowing and the younger plants collapse but older ones turn yellow and may dry without collapsing. The younger plants exhibit clear rotting at the collar region. The rotten portion is often covered with white mycelial strands of *S. rolfsii*. Looking towards the above facts an attempt was made to study the Efficacy of bio-agents and organic amendments against *Sclerotium rolfsii*.

MATERIAL AND MATHOD

Seed treatment

Seed treatment was done with *Trichoderma* @ 4 gm/kg seeds in T1. In treatment T2, T3 and T4 neem cake, mustard cake and karanj cake respectively was mixed in the soil and seeds were not treated with *Trichoderma*. Treatment T5, T6 and T7 received seed treatment with *Trichoderma* and soil treatment with neem cake, mustard cake and karanj cake respectively, was done. No seed treatment and soil treatment served as control.

Seedling inoculation

In seedling stage, test fungus was inoculated at the collar region of plants in two lines of each plot and then irrigated lightly by hand shower and mortality were recorded 15 days after inoculation.

Experimental details

Season	:	Rabi – 2007
Situation	:	Upland and Kanhar (Vertisol)
Design	:	Randomized Block Design (RBD)
Replications	:	Three
Treatments	:	Eight
Variety	:	JG-315
Plot size	:	2 x 3 m^2
Seed rate	:	80 kg/ha
Date of sowing	:	7 November, 2007

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Details of Treatments

S. N.	Treatment	Notation
1	*Trichoderma* spp	T1
2	Neem cake	T2
3	Mustard cake	T3
4	Karanj cake	T4
5	*Trichoderma* spp + Neem cake	T5
6	*Trichoderma* spp + Mustard cake	T6
7	*Trichoderma* spp + Karanj cake	T7
8	Control	T8

Observation recorded

1. Total plant population per plot
2. Mortality were recorded after 15, 30, and 45 days of sowing.
3. Yield of plots.

RESULT AND DISCUSSION

Efficacy of bio-agent with organic amendments against plant mortality caused by *S. rolfsii* (under natural condition)

Results (Table1) revealed that all the organic amendment significantly increased seed germination and reduced collar rot incidence over untreated control. However, among all treatments, seed treatment of bio-agent *Trichoderma* with Neem cake soil application was found to be the most effective recording maximum seed germination (93.05%) and minimum total mortality (8.32%) followed by *Trichoderma* with mustard cake (85.03 & 9.55%) and *Trichoderma* with karanj cake (80.86 & 11.41%).

Efficacy of bioagent with organic amendments against plant mortality caused by *S. rolfsii* (under inoculated condition)

After completion of all observations two lines of each treatment were artificially inoculated by test fungus *Sclerotium rolfsii* in all three replications. The results (Table 2) revealed that among all treatments, seed treatment of bio-agent *Trichoderma* and soil application with Neem cake found to be most effective with maximum germination (96.29%) and least total mortality (18.51%), followed by *Trichoderma* and Mustard cake (92.59%) and mortality per cent (25.92) and *Trichoderma* with Karanj cake (92.59%) and mortality per cent (25.92). These treatments were statistically at par with other for germination and total mortality. Upmanyu *et al*. (2002) reported that soil amendment with cotton, mustard and neem cakes were effective in reducing pre and post emergence incidence of root rot of frenchbean under glasshouse and field condition. Prasad *et al*. (1999) reported that isolates of *Trichoderma* and *Gliocladium* sp. inhibited mycelial growth (54.9 to 61.4%) and suppressed the sclerotial production (31.8 to 97.8%) of *S. rolfsii*, the causal organism of root and collar rot of sunflower *in-vitro*. Bhoraniya *et al.* (2003) found that castor oil cakes reduced the stem rot disease (caused by *Sclerotium rolfsii*) incidence of chilli by 78.57 per cent, while sesame oil cake against foot rot of brinjal (Siddique *et al*., 2002). Similarly, neem cake against wilt of bell pepper (Chowdary *et al.* 2000) and mustard cake against wilt of potato (Baker and Khan, 1981) were superior in controlling the disease caused by *S. rolfsii* in pot culture.

Table 1. Effect of bio-agent with organic amendments, on plant mortality under natural conditions

S. No.	Treatment	Germination* (%)	Mortality*(%)			Total mortality (%)
			15 DAS	30 DAS	45 DAS	
1	*Trichoderma* sp.	72.99 (58.66)**	2.46	5.55	6.01	14.02 (2.24)***
2	Neem cake	66.82 (54.87)	2.77	5.55	5.40	13.72 (2.23)
3	Mustard cake	66.97 (55.18)	3.24	5.86	6.17	15.27 (2.35)
4	Karanj cake	61.88	3.39	5.40	5.40	14.19

		(51.91)				(2.27)
5	*T*. sp with neem cake	93.05 (74.82)	1.54	3.24	3.54	8.32 (1.79)
6	*T*. sp with mustard cake	85.03 (67.80)	2.00	3.54	4.01	9.55 (1.90)
7	*T*. sp with karanj cake	80.86 (64.30)	1.85	5.09	4.47	11.41 (2.04)
8	Control	57.40 (49.47)	4.62	9.41	9.25	23.28 (2.84)
	SEm ±	0.40	3.05	2.74	3.32	0.05
	CD (p=0.05)	1.22	8.45	7.61	9.20	0.17

*=Average of three replications
DAS= Days after sowing
**=Data in parenthesis show Arc sine transformation
***=Sq. root transformation value in parenthesis

Table 2. Effect of bio-agent with organic amendments on collar rot under artificially inoculated conditions

S. No.	Treatments	Germination* (%)	Mortality* (%)		Total mortality (%)
			L1	L2	
1	*Trichoderma* sp.	85.18 (68.67)**	14.81	14.81	29.62 (3.91)***
2	Neem cake	85.18 (67.44)	18.51	22.22	40.74 (4.56)
3	Mustard cake	81.48 (63.72)	25.92	29.62	55.54 (5.31)
4	Karanj cake	74.07 (59.40)	22.22	29.62	51.86 (5.12)
5	*T*. sp with neem cake	96.29 (77.30)	7.40	11.11	18.51 (3.10)
6	*T*. sp with mustard cake	92.59 (74.51)	14.81	11.11	25.92 (3.66)
7	*T*. sp with karanj cake	92.59 (74.51)	11.11	14.81	25.92 (3.66)
8	Control	48.15 (43.91)	33.33	37.03	70.37 (5.97)
	SEm ±	2.34	0.20	0.60	0.19
	CD (p=0.05)	7.12	0.56	1.67	0.65

*= Average of three replications
**=Data in parenthesis show Arc sine transformation

***=Sq. root transformation value in parenthesis
L1= Line no. 1
L2= Line no. 2

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RESEARCH ARTICLE

INSECT PESTS COMPLEX ASSOCIATED WITH BASMATI RICE WITH WESTERN PLAIN ZONE OF UTTAR PRADESH, INDIA

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Abstract: Insect pests complex associated with basmati rice were studied during *Kharif*, 2014 and 2015 at Crop Research Center of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. During the study period, fifteen insect species were encountered on basmati rice in western plain zone of Uttar Pradesh which belong to 7 orders *viz*. lepidoptera (yellow stem borer, leaf folder, striped rice stem borer, rice case worm and swarming caterpillar), homoptera (green leaf hopper, brown plant hopper, and white backed plant hopper), heteroptera (rice gundhi bug), hetroptera (rice mealy bug), coleoptera (rice root weevil and white grub), isoptera (termite) and orthoptera (*Kharif* grass hopper and grass hopper).

Keywords: Insect pests, Basmati rice, Grass hopper

INTRODUCTION

Rice is the major food of the largest population of the world. About 90 per cent rice in the world is grown and consumed by the population of the Asian countries (Samanta, 2014). In India, it occupies an area of about 43.95 million hectare with total production of 106.54 million tones and productivity of 2.4 tones per hectare (Anonymous 2014). Basmati rice crop suffers severely due to attack of various insect pests which reduces its yield and quality. More than 300 species of insects have been reported to attacked rice crop from the germination of nursery till its harvests (Jadhao and Khurad, 2011). In general, yield loss due to insect pest of rice has been estimated at about 25 per cent in different rice ecosystem (Sachan *et al*., 2006 and Dhaliwal *et al*., 2010). Keeping in view this fact, the present investigation to update the information about insect pests complex associated with basmati rice in this region.

MATERIAL AND METHOD

An experiment were conduct during two consecutive crop season, *viz Kharif*, 2014 and 2015 at Crop Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U. P.). The rice cultivar PB-1 was sown during mid of June and transplanted in the second week of July in both the years and adopted recommended practices to rice good crop. Observations on insect pests associated with rice were recorded from the germination of seedlings till the harvest of crop at weekly interval. The insect were collected and identified.

RESULT AND DISCUSSION

During the period of study, 15 insect species belonging to 7 orders were recorded on basmati rice at different crop growth stage. Among them, yellow stem borer, *Scirpophaga incertulas* Walker and leaf folder, *Cnaphalocrocis medinalis* Guenee were found as major pests. The brown plant hopper, *Nilaparvata lugens* Stal., white grub, *Holotrichia consanguinea* Blanch, termite, *Odontotermes obesus* Romb. and *Kharif* grass hopper, *Hieroglyphus banian* Fab. were found moderately damaging the crop. The rice swarming caterpillar (army worm), *Spodoptera mauritia* Boisduval, striped rice stem borer, *Chilo suppressalis (*Walker*)*, rice case worm, *Nymphula depunctalis* (Guenee), green leaf hopper, *Nephotettix virescens* Distant, white backed plant hopper, *Sogatella furcifera* Horvath, rice gundhi bug, *Leptocorisa acuta* Thunb, rice root weevil, *Echinocnemus oryzae* Marshall, rice mealy bug, *Brevennia rehi* (Lind.) and grass hopper, *Oxya fuscovittata* Marshall recorded on the crop were of less importance and extent of their damage was found without much economic loss (Table 1).

Yellow stem borer is the most destructive and dominant species of rice crop in this region and is reported to occur throughout the country and other Asian countries. The appearance of pest started in the beginning of July and remained active throughout the crop season. The newly hatched larvae enter in leaf sheath and bore into the stem near the node and causing death of central shoot ‘dead hearts’ in vegetative stage and ‘white ear head’ at milky stage, respectively. This resulted in chaffy grains. The severity of this pest was recorded from beginning of August to September end. The damage of yellow stem borer on rice crop has also been reported by Kumar and patil (2004), Dogra and Amit (2005), Satpathi *et al.* (2012) and Gangwar *et al*. (2015). Sachan *et al.* (2006) also reported the severe

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incidence of *S. incertulas* on basmati rice throughout the crop season in tarai region of Uttar Pradesh.

Rice leaf folder is another major pest of basmati rice recorded from middle of August to September end. It has inflicted severe losses on rice crop. The newly hatched larvae crawl to the base of the youngest unopened leaf and begin to feed and then migrate to an older leaf and folds leaf together. Under heavy infestation reduced the general vigor and photosynthetic ability of an infested plant. Considerable losses to paddy crop due to leaf folder have been reported by several workers like Sachan *et al*. (2006), Jadhao and Khurad (2011) and Saini *et al.* (2015).

The striped stem borer is a polyphagous pest frequently occurring in the rice fields of almost all fields in the area. The first symptoms of damage were the drying off of growing points and the surrounding leaves and finally, leaves fall off. In the infested field white heads stand erect and contain empty and unfilled glumes. The striped yellow stem borer on rice crop has also been reported by Pathak (1968), Singh and Singh (2014) and Gangwar *et al*. (2015) reported that important insect pest of rice in India and South- East Asia.

The swarming caterpillar is considered to be a sporadic pest which occasionally causes serious losses to upland rice crop. Larvae of this pest fed on the upper portion of rice canopy by defoliating leaves during night. This insect recorded as minor pest in the month of July –August. This swarming caterpillar on rice crop has also been reported by several workers like Jadhao and Khurad (2011), and Gangwar *et al*. (2015).

The rice case worm is an important insect pest of rice. The damaging stage is the larvae that live in sections of leaves cut from young rice plants and rolled tubes called cases. Rice at seedling and tillering stages are the preferred host but does not occur after maximum tillering. The insect was recorded as minor pest of this region. The damage of this insect has also been reported by Dale (1994), Singh and Singh (2014) and Gangwar *et al*. (2015). This pest is widely distributed in rice growing countries of Asia.

The incidence of green leaf hopper, *Nephotettix virescens* (Distant) was recorded in August-September. Both, nymphs and adults of this insect sucked sap from the leaves and tender parts of plants by turning them yellow. Brown plant hopper, *Nilaparvata lugens* (Stal) is another important pest of rice. Its infestation was recorded from middle of August to September end. As a result of feeding by both nymphs and adults at the base of the tillers, plants turns yellow and dry up rapidly. At early infestation round yellow patches appeared which soon turn brownish due to the drying up of the plants. This condition is called "hopper burn". Complete destruction of the crop was recorded in severe cases. White backed plant hopper, *Sogatekka furcifera* (Horvath) was recorded in August - September as minor pest in this region. It sucked the sap from tender leaves, thus causing yellowishness of them. The honeydew produced by the hoppers serves as a medium for growth of sooty mould.

The damage caused by green leaf hopper, brown plant hopper, and white backed plant hopper on rice crop has been reported by various workers such as Sachan *et al*. (2006) and Singh and Singh (2014). However the finding of Atwal and Dhaliwal (2005), Srivastava (2006), Sachan *et al*. (2006), Singh and Singh, (2014) and Sharma (2015) are also in conformity with present finding that green leaf hopper and brown plant hopper were the pest of basmati rice.

The rice gundhi bug was recorded as important pest of rice crop in this region. Both nymphs and adults sucked the sap of developing grains during milky stage and thus make them chaffy. Whole panicle becomes white colored (chaffy) under severe infestation. Its occurrence was recorded during September–October. Atwal and Dhaliwal (2005), Sachan *et al*. (2006), Kashyap (2013), Singh and Singh, (2014), Gangwar *et al*. (2015) and Sharma (2015) also reported the damage of this pest during September-October on rice crop.

Rice mealy bug is a polyphagous pest. The mealy bug occurs in colonies attached to the stem and leaf sheaths of plants. They sucked the sap from the plant. The high incidence of this pests inhibits panicle emergence and plant may even dry. This pest first time reported in this region. Singh and Singh (2014) has been reported incidence of rice mealy bug in northeastern Uttar Pradesh.

Rice root weevil was also recorded a common pest of basmati rice at vegetative stages. Grubs fed on the roots and rootlets of young rice plants, resulted in stunting and non formation of tillers. The leaves turn yellow and develop a rusty appearance and the plants eventually die. Incidence of this pest was noticed in the month of July and August. Singh and Singh (2014) and Gangwar *et al*. (2015) also reported the occurrence of this pest on rice crop.

White grubs are a serious polyphagous pest and damage almost all the *Kharif* crops from June to October. The young larvae feed upon roots and decaying vegetation throughout the summer and, in autumn, migrate downward (to a depth of up to 1.5 meters) and remain inactive until the following spring. The severe damage occurs as the larvae move near the soil surface to feed on the roots of the plants. The beetles overwinter in the soil, emerging the following year in May or June when feeding, mating, and egg-laying take place. The insect was recorded from July to October *i.e*. throughout the crop season. Atwal and Dhaliwal (2005), Kashyap (2013) and Saini (2015) also reported the incidence of this insect from July to October on various *Kharif* crops.

Termite is a polyphagous social insect, also caused damage to the rice crop by feeding on the roots of the

plants. The growing shoots withered and died. The damaged plants pulled out easily. The incidence of this pest was observed throughout the crop season. Such type of damage has earlier reported on different crops by Atwal and Dhaliwal (2005), Prasad and Prasad (2006), Sachan *et al.* (2006), Srivastava (2006), Kashyap (2013), Singh and Singh (2014) and Saini (2015).

The two species of grasshoppers namely *Hieroglyphous banian* (Fab.) and *Oxya fuscovittatta* (Marshall) were found to attack basmati rice in this region. Nymphs and adults of grasshoppers fed on the leaves by making holes and were found active throughout the crop season while latter was found in the month of August and September as minor pest. Both these species of grasshopper are polyphagous pest and have earlier been reported by Sachan *et al*. (2006), Prasad and Prasad (2006), Kashyap (2103), Singh and Singh (2014), Sharma (2015), Gangwar *et al*. (2015) and Saini (2015) on paddy crop. Usmani *et al.* (2012) observed *Kharif* grass hopper as a pest of paddy from central Uttar Pradesh.

Table 1. Insect pests complex associated with basmati rice during *kharif* 2014 and 2015

Common Name	Scientific Name	Order - Family	Damaging stage of the pest	Severity of the pests
Yellow stem borer	*Scirpophaga incertulas* (Walker)	Lepidoptera : Pyralidae	Larvae	Severe
Striped rice stem borer	*Chilo suppressalis (*Walker*)*	Lepidoptera : Pyralidae	Larvae	Low
Leaf folder	*Cnaphalocrosis medinIalis* (Guenee)	Lepidoptera : Pyralidae	Larvae	Severe
Swarming caterpillar (Army worm)	*Spdoptera mauritia* (Boisduval)	Lepidoptera :Noctuidae	Larvae	Low
Rice case worm	*Nymphula depunctalis* (Guenee)	Lepidoptera: Pyralidae	Larvae	Low
Green leaf hopper	*Nephotettex virescens* (Distant)	Homoptera :Cecadellidae	Nymphs and adults	Low
Brown plant hopper	*Nilaparvata lugens* (Stal)	Homoptera : Delphacidae	Nymphs and adults	Moderate
White Backed plant hopper	*Sogatekka furcifera* (Horvath)	Homoptera : Delphacidae	Nymphs and adults	Low
Rice gundhi bug	*Leptocorisa acuta* (Thumb)	Hetroptera :Coreidae	Nymphs and adults	Low
Rice mealy bug	*Brevennia rehi* (Lind.)	Hemiptera:Pseudococcidae	Nymphs	Low
Rice Root Weevil	*Echinocnemus oryzae* (Marshall)	Coleoptera : Curculionidae	Grubs and adults	Low
White grub	*Holotrichia consanguinea* (Blanch)	Coleoptera : Curculionidae	Grubs and adults	Moderate
Termite	*Odontotermes obesus* (Romb)	Isoptera : Termitdae	Worker	Moderate
Kharif grass hopper	*Hieroglyphus banian* (Fab)	Orthoptera : Acrididae	Nymphs and adults	Moderate
Grass hopper	*Oxya fuscovittata* (*Marshall*)	Orthoptera : Acrididae	Nymphs and adults	Low

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SHORT COMMUNICATION

PLANTS AS A SOURCE OF DIURETIC ACTIVITY AND STUDY OF 3-(6-ARYLIMIDAZO[2,1-B]THIAZOL-3-YL)-2-METHYLCHROMONE SYSTEM AS DIURETIC AGENT

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Abstract: Diuretic agents increase urine volume and are effective in heart failure , renal failure and maintain Na^+ ion balance. They are also effective in hypertension and nephrosis. Though plants possess diuretic activity , but their delayed action needs to use quickly acting agents . In this paper study on 3-(6-Arylimidazo[2,1-b]thiazol-3-yl)-2-methylchromones as diuretic agents is being discussed . Lead for diuretic activity has been found in this system .

Keywords: Diuretic activity, Chromones, Lead, Structure activity relationship (SAR)

INTRODUCTION

Diuretics are the drugs that increase urine volume as well as flow and clinically useful diuretics increase the rate of excretion of Na^+ ions (natriuresis) and accompanying ions like Cl^- ions (Jackson, 2001) . They adjust urine volume and composition of body fluids in situations like hypertension, heart failure, renal failure , nephrosis etc. (Jackson, 2001) . Most of the diuretics have adverse effects like fatigue, weakness and impotence. Plant based diuretics include caffeine present in tea, coffee and cola which inhibit sodium ion re-absorption . Most of the diuretics are effective in promoting sodium ion excretion; but all cause potassium ion loss (Vanamala *et al.* , 2012) . Moreover, resistance develops to diuretics (Brater, 1983). More than 650 herbal preparations in the form of tablets, decoctions, tinctures etc. have shown diuretic activity (Chopra *et al.*, 1986). *Achyranthes aspera* Linn .also possess diuretic activity (Srivastava *et al*., 2011). Aqueous extract of mango bark (*Mangifera indica*) is also diuretic (Shree Devi , 2011). Kane et al. (2009) reported potentiation of diuretic activity through ethanolic extract of *Euphorbia thymifolia*. Thus, various plant parts are good diuretic agents (Dutta et al. , 2014) ; but their action is delayed . Therefore, there is need to develop other diuretics which are non-resistant and non-toxic. Probably diuretic activity in plants is due to the presence of flavonoids and alkaloids in them (Vanamala et al., 2012) . Few chromone derivatives have shown diuretic activity in past (Sharma, 2015). Hence, it was thought to study diuretic activity in 3-(6-Arylimidazo[2,1-b]thiazol-3-yl)-2-methylchromones .

MATERIAL AND METHOD

3-(6-Arylimidazo[2,1-b]thiazol-3-yl)-2-methylchromones were synthesized by author in the Department of chemistry , Kurukshetra University , Kurukshetra (Garg et al. , 1985 ; Sharma , 2005) . The diuretic activity was tested at CDRI , Lucknow . Activity of compounds was compared with Chlorothiazide standard (value for which is taken as 100) [Table-1] .

RESULT AND DISCUSSION

6-Chloro-3-(6-phenylimidazo[2,1-b]thiazol-3-yl)-2-methylchromone (VPS-10) showed diuretic activity equal to 100 which is same as for chlorothiazide standard . However, compound is a bit toxic with ALD_{50} = 681 . VPS-11 with two halogens (chlorine atoms) , one at C_6 of chromone ring and other at *p*-position of phenyl ring present at C_6- position of imidazothiazolyl moiety , shows decrease in activity which becomes equal to 93 . This compound becomes safer [ALD_{50} > 1000] . Here one additional chlorine atom at conjugated position of phenyl ring decreases activity and toxicity as well. VPS-12 has one additional methyl group compared to VPS-10 at C_7- position of chromone ring; it exhibited less activity than VPS-10. It is inferred that additional methyl groups result in reduction of diuretic activity (*c. f.* Gupta , 2014) . One additional methyl group also reduced toxicity as ALD_{50} changes from 681 to 1000.

VPS-13 has two halogen atoms , Cl at C_6- position of chromone moiety and Br at *p*- position of phenyl ring present at C_6 – of imidazothiazolyl moiety . This compound also possesses a methyl group at C_7 – of chromone ring. Here reduction in diuretic activity is due to substituent Br and methyl group . Therefore , activity is much reduced and becomes equal to 68 because of the additive effect of Br and CH_3 . Toxicity of this compound is low because both Br and CH_3 decrease toxicity. 3-(6-(*p*)-chlorophenylimidazo[2,1-b]thiazol-3-yl)-2,6-dimethylchromome (VPS-14) has intermediate activity of 87 as methyl group decreases the activity and Cl substituent increases the activity in comparison to methyl group. As both these

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substituent decrease the toxicity the compound is safer for use with ALD_{50} value of 1000.

A look at these activities shows that additional halogen atom either Cl or Br decreases activity to same extent [activity of VPS-10 > VPS-11 by 7-units and activity of VPS-12 is > VPS-13 by 8-units] which may be ascribed to their same electronic effects (Silverman , 2004). Replacement of Cl by CH_3 group decreases the activity by 6-units [*c.f.* activity of VPS-11 and VPS-14].

Table 1. Effect of substitution on the diuretic activities of 3-(6-arylimidazo[2,1-b]thiazol-3-yl)-2-methylchromones :

S.No.	Code	Name of the compound	Activity	ALD_{50}
1.	VPS-10	6-chloro-3-(6-phenylimidazo[2,1-b]thiazol-3-yl)-2-methylchromone	100	681
2.	VPS-11	6-chloro-3-(6-(*p*)-chlorophenylimidazo[2,1-b]thiazol-3-yl)-2-methylchromone	93	>1000
3.	VPS-12	6-chloro-3-(6-phenylimidazo[2,1-b]thiazol-3-yl)-2,7-dimethylchromone	76	1000
4.	VPS-13	6-chloro-3-(6-(*p*)-bromophenylimidazo[2,1-b]thiazol-3-yl)-2,7-dimethylchromone	68	1000
5.	VPS-14	3-(6-(*p*)-chlorophenylimidazo[2,1-b]thiazol-3-yl)-2,6-dimethylchromone	87	1000
6.	Standard	Chlorothiazide	100	Drug

CONCLUSION

Methyl group at C_6 and C_7 – positions of chromone ring decreases the activity and introduction of halogen atom at *p*- position of aryl group present at 6-position of imidazothiazolyl moiety of 3-(6-arylimidazo[2,1-b]thiazol-3-yl)-2-methylchromones though decreases the activity but increases the diuretic activity in comparison to methyl group which may be attributed to their electronic effects . As all the tested 3-(6-arylimidazo[2,1-b]thiazol-3-yl)-2-methylchromones exhibited good diuretic activity so this system is a lead for this activity .

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