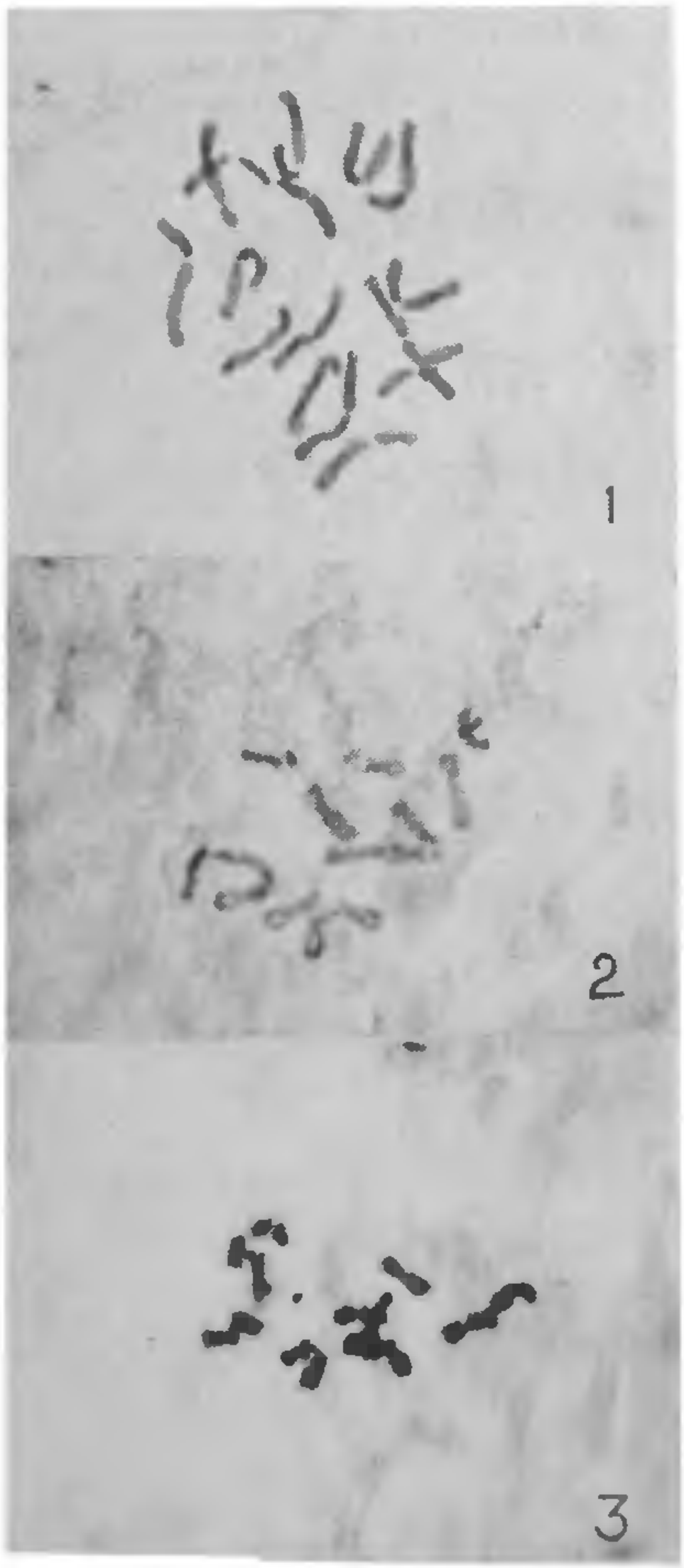
boxylic acid)—the most active morphactin tested so far[2], it was found that the compound showed C-mitotic effects when applied to fast growing roots[3]. It was therefore desired to test this morphactin as a pretreating agent for chromosome analysis.



Figs. 1–3. Somatic metaphase spreads of Fig. 1. *Allium cepa*, Fig. 2. *Lathyrus sativus*, Fig. 3. *Lens culinaris*.

Using a wide variety of plants, belonging to different families having small to large sized chromosomes with low to high number of chromosomes, it has been observed that the chemical can successfully be used for cytological purpose as a pretreating agent. It brings about metaphase arrest by destruction of spindle with the chromosomes remaining free, clearing of cytoplasm and causes differential hydration of chromosome segments. Pressure applied during smearing of root tips, results in well scattered metaphase plates with constriction regions in chromosomes well clarified. The pretreatment schedule is performed as follows :

Fast growing root tips are transferred to an aqueous solution of *Chlorfluerenol* (0·03%) and then given a short (5 minutes) chilling treatment in ice chamber. The material is then kept for about 3–3½ hrs at 12–16° C. Root tips are thoroughly washed in water and fixed in suitable fixative. Fixed roots are processed as usual for cytological preparations.

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ESTIMATION AND IDENTIFICATION OF ALKALOIDS PRODUCED BY *CLAVICEPS FUSIFORMIS* LOVELESS ON SOME VARIETIES OF PEARL MILLET

BAJRA, the pearl millet (*Pennisetum typhoides* S and H) is one of the most important rainy season crops of the arid and semi-arid areas of India. This also provides a staple cereal diet to the people residing in the rural parts of the country. Last few years it was observed that pearl millet was heavily infected with the ergot disease, caused by *Claviceps fusiformis* Loveless all over India[1]. The ergot menace is particularly more pronounced on the improved and high yielding hybrid varieties of bajra. In severe form it has been estimated to cause grain losses of about 58 to 75% with 62% disease incidence[2].

Various reports are on record concerning with the poisoning in humans and animals due to the consumption of ergot contaminated bajra from Rajasthan and Maharashtra States[3,4]. The common symptoms of ergot poisoning include giddiness, diarrhoea, nausea and vomiting followed by dehydration. An agalactia disease in sows due to ergoty bajra has also been reported from Rhodesia. The cause seems to be the fungal alkaloids[5]. The alkaloids are suspected to belong to clavine group instead of ergotoxine ergotamine and ergometrine groups, characteristic of *Claviceps purpurea* (Fr.) Tul., the causal agent of ergot of wheat and rye[4],

A marked influence of host species on the production of alkaloids has been suggested due to the host's nutrient status[6]. The present paper deals with the identification and quantitative determination of total alkaloid contents of honey-dew and sclerotia obtained from different varieties of bajra infected with ergot fungus.

Twenty cultivars including hybrid and local varieties were raised in the field of University botanical garden. The fungus was isolated on Murashige and Skoog (MS) medium, using ripe sclerotia obtained from HB-4 (new) variety. The fungus was purified by raising monoconidial cultures which were maintained on solid synthetic CNM (calcium nitrate agar) medium. The usual method followed for infection was to atomize aqueous spore suspension prepared in sterile distilled water in the floral spike immediately after its emergence from the boot. In all four sprays were given on alternate days and the spikes were covered with polythene bags immediately after inoculation. Alkaloid contents of honey-dew and sclerotia were analysed on tenth and twentieth day, respectively after inoculation.

TABLE I

Alkaloid content of honey-dew and sclerotia (values expressed as elymoclavine equivalents)

Sl. No.	Variety	Total alkaloid contents (in per cent)	
		Honey-dew	Sclerotia
1.	Senegal dwarf	0·182	..
2.	A–836	0·264	..
3.	B–463	..	0·548
4.	HB–4 (new)	..	0·204
5.	R–342	0·244	0·224
6.	R–360	0·300	0·296
7.	Jakrana local	0·320	..
8.	R–221	..	0·344
9.	B–389	0·322	..
10.	Jodhpur local	..	0·364
11.	J–88	..	0·245
12.	B–197	0·364	0·208
13.	126 D_2B	0·256	0·208
14.	B–305	0·232	0·300
15.	R–339	..	0·204
16.	R–357	0·280	..
17.	MPP–7632	0·264	..
18.	R–117	0·208	..
19.	RC–216	..	0·160
20.	R–417–1	0·192	..
	Mean	0·263	0·275

The total alkaloid contents of honey-dew and sclerotia were determined quantitatively by modified Mukerji and De's method[7]. Per cent alkaloid contents were calculated by comparing with the readings obtained with elymoclavine standard sample.

The identification of alkaloids was made by thin layer chromatography using glass plates (20 × 20 cm) pre-coated with silica-gel-G. The plates were developed with chloroform : dimethyl formamide : acetone : ethyl alcohol (6 : 2 : 1 : 1 V/V). The blue coloured spots of alkaloids were detected by spraying van Urk's reagent.

The fungus produced high amount of alkaloids both in honey-dew and sclerotia. The total alkaloid contents in different bajra varieties were found in between 0·182 to 0·364 per cent with an average of 0·263 per cent in honey-dew. 0·160 to 0·548 per cent range was observed in sclerotia (Table I). Among the twenty varieties tested, maximum alkaloid production (0·548%) was observed in variety B-463, while the minimum production (0·160%) was recorded in variety RC-216. A considerable variation in the alkaloid contents of honey-dew and sclerotia was observed. For the identification of the alkaloids present both in honey dew and sclerotia, pure mixtures of elymoclavine, agroclavine, penniclavine, chanoclavine, setoclavine and ergometrine maleate B. P. were employed. The alkaloids were detected on the basis of co-chromatography and their Rf values (Table II). Ergometrine (Rf 0·19) was not observed in the samples tested.

TABLE II

Identification of alkaloids present in pearl millet ergot

Colour reaction with van Urk's reagent	Rf	Identification
Blue	0·94	Setoclavine
Blue	0·74	Agroclavine
Bluish green	0·70	Penniclavine
Blue	0·45	Elymoclavine
Green	0·29	Unidentified
Blue	0·17	Chanoclavine

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ROLE OF THIAMINE HYDROCHLORIDE AS A HATCHING FACTOR IN LARVAL EMERGENCE FROM CYSTS OF *HETERODERA ORYZICOLA* (NEMATODA: HETERODERIDAE) ON RICE

THIAMINE hydrochloride (3 mM), undiluted rice root diffusate and flavionic acid (3 mM) stimulated the hatching of larvae from cysts of *Heterodera oryzicola* n.sp. While the chain legnth between the two terminal polarisable atoms of flavionic acid was the hatching factor, the effectiveness of thiamine hydrochloride with only one polarisable atom was through the enzyme system. Seed coat and roots of rice are rich in thiamine which along with the root diffusates stimulate hatching continuously in soils with monoculture of rice.

Root diffusates of plants[1,2], certain dyes[3] and metallic ions like zinc[4,5] stimulate the emergence of larvae of *Heterodera schachtii*. Organic compounds, *viz.*, anhydrotectronic acid[6], picrolonic acid[7] against *Globodera rostochiensis*, flavionic acid[8,9] against several *Heterodera* spp. and nematicides like nabam (sodium ethylene bis dithiocarbamate)[10] against *H. schachtii* at concentrations of 0·6 to 3 mM proved effective as hatching agents. Some of these along with some constituents of vitamin B and undiluted diffusates from roots of rice c.v. CRM13-3241 (NSJ 200 × *Padma*) were tested against the cysts of *H. oryzicola* n.sp.[11] by the methods described earlier[4,5,10].

Hatching was significantly high in thiamine hydrochloride (vitamin B_1) followed by rice root diffusate and flavionic acid (Table I). Pyridoxine, folic acid and sodium metavanadate were moderately active. The other vitamin constituents, dyes and zinc salts were inactive. Larvae emerging from thiamine hydrochloride treatment were active and infective like those emerging from the rice root diffusate. Hatching agents stimulated the coiled larva inside the egg and the emerged larva was active upto 72 h until penetration into rice roots suggesting that it has the energy reserves for motility but lacked stimulus for breaking the dormancy while still in egg.

TABLE I

Emergence of larvae from cysts of Heterodera *oryzicola n.sp. soaked in test compounds at conc. 3 mM (mean cumulative hatch of 3 batches of 50 cysts each during 21 days)*

Compound	Hatch rating*
Thiamine hydrochloride	123
Pyridoxine	62
Folic acid	40
Riboflavin	2
Calcium pantothenate	1
Nicotinic acid	1
Rice root diffusate (undiluted)	100
Flavionic acid	92
Picrolonic acid	1
Sodium metavanadate	33
Zinc chloride	5
Zinc sulphate	2
Auramine	2

* Based on Clarke and Shepherd[8].

The role of thiamine hydrochloride as a co-enzyme with pyruvic acid dehydrogenase in the further metabolism of the energy rice pyruvic acid in animals has been established[12,13]. The effectiveness of thiamine as a hatching factor in *H. oryzicola n. sp* suggests that the dormancy had been effected by blocking the intermediary metabolism. Unlike in the other cyst nematodes responding to flavionic acid[8] where the chain length between the two terminal polarisable atoms was the factor influencing hatching, thiamine hydrochloride with only one polarisable atom was also found to be efficient as a hatching stimulant. Though the role of flavionic acid in influencing the emergence of larvae from cysts through the enzyme system has not yet been established, the effectiveness of thiamine is considered to be through the enzyme system.

Most enzymes of Glycolytic and TCA cycles in nematodes have been demonstrated[14] and homogenates of the nematode *Ditylenchus triformis* were able to utilise labelled glucose and pyruvate and convert them into CO_2[15]. Seed coat and roots of rice are rich in thiamine and the present study confirms that the root diffusates and these may effect hatching of cysts of *H. oryzicola* n. sp through thiamine *via* the enzyme system. Due to the above reason, hatching was