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Semi quantitative estimation of prussic acid (HCN) in some fodder plants of Nanded district of Maharashtra state

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Abstract
Cyanide poisoning in cattle's may occur due to grazing of cyanogenic plants. HCN is known to occur in at least 2700 plants in the world in the form of cyanogenic glycosides. It is produced after enzymatic hydrolysis of the cyanogenic glycoside. It is considered to be a defence mechanism of plants against pests and its production in plants depends on number of ecological and physiological conditions. In the present study HCN content in 13 fodder plants from 7 families were analyzed. The semi quantitative estimation of HCN is done with the help of a simple kit. 12 fodder plants were tested positive for HCN.

Keywords: HCN Cyanide content and fodder plants

Introduction
Cytogenesis is the ability of some plants to synthesize cyanogenic glycosides, which when enzymically hydrolyzed, release cyanohydric acid (HCN) known as prussic acid (Harborne, 1972, 1986) [4, 5]. The word 'cyanide' can stir people's emotions. Cyanide C ≡ N exists in various forms in nature as salts of a potassium, sodium and calcium. These are known to be most rapid potent highly poisonous substances in the world, but they are very important in the process of gold recovery from hard rock and extensively used industrially. Cyanide and chemically related compounds are formed, excreted and degraded in nature by hundreds of species of bacteria, algae, fungi and higher plants (Knowles, 1976) [7].
At least 2600 species of higher plants and some microorganisms have been shown to contain one or more of nearly thirty two compounds capable of producing hydrogen cyanide (HCN) or prussic acid. These compounds are mostly derived from amino acids (Seigler, 1976, Moller and Seigler 1999) [12, 8]. Among the higher plants at least 2600 are known to be cyanogenic. The process of cyanogenesis is very simple. The cyanogenic glucoside present in the plants under some conditions is converted to sugar and an algycone with the help of b-glycosidase enzyme. In the next step with the help of enzymes the aglycone is converted to HCN and an aldehyde or ketone with the help of another enzyme. Poisoning of live stock by forage Sorghum and other plants is well documented (Mudder, 1997) [9]. Occasional accidental poisoning in humans have also been reported (Pentore *et al.* 1996) [11]. There are many economical important plants highly cynogenic, including white clover, linum, almond, sorghum, the rubber tree and cassava (Tokarnia *et al*., 1994; Cheeke, 1995) [13, 2]
In the present study an attempt has been made to detect and estimate amounts of HCN semi quantitatively from some fodder plants so as to understand the risk factor of consuming these plants by animals and which is highly used against number of ailments in rural areas by human beings.

Materials and Methods
All 13 fodder plants were collected from different regions of Marathwada particularly Nanded and adjoining District and was immediately identified botanically on the spot in the field by using Flora of Marathwada (Naik, 1998) [10].
After pressing and drying herbarium sheets of these plants are prepared. Field tests for cyanogenic plants were taken wherever possible and even in some cases quantitative estimation was done by the simple picrate paper kit of Bradbury (Bradbury *et al*., 1999) [1]. All the chemicals and reagents used were purchased from the commercial sources and were of analytical grade.

Semiquantitative estimation of HCN content
Fodder plants were tested for the presence of cyanogenic glycosides and release of HCN by

simple sodium picrate paper test. The leaf/fruit extract suspected for the presence of cyanogenic compounds were taken in 0.2 M. phosphate buffer pH 10 ml was added. A strip of Whatman filter paper No.1, 5cm x 2cm. was soaked in sodium picrate solution (25gms of sodium carbonate and 5gms of picric acid dissolved in 1000 ml of distilled water) and dried, and it was hanged in glass vials containing the extract of selected fodder plants to be tested. The colour of the picrate paper was observed after few hours. If the colour changed from yellow to reddish brown, it indicated the presence of HCN in the plant extract and test was positive. Intensity of colour change is related to the amount of the cyanogens present and it is possible to observe colour rating as a measure of concentration. If the test is negative, the tube should be left at room temperature for further 24-48 hours and then re-examined for any non-enzymatic release of HCN.

The quantitative estimation of total cyanide in plant materials is a complicated process. However a simple method and kit for semi-quantitative estimation of HCN is developed by Egan *et al*. (1998) and Bradbury *et al*. (1999) [3, 1]. This kit make use of filter paper caps of enzyme linamarase for release of HCN from glycoside but in the present study instead of it specially prepared caps of filter paper soaked in extract of *Cuscuta reflexa* Roxb were used as source of b-glycosidase enzyme. The colour change is compared with the standard colour chart provided by Bradbury, which gives an approximate amount of HCN in ppm.

Table 1: Detection and estimation of hydrogen cyanide in some fodder plants by using certain simple techniques

S. N.	Name of plants	Family	Common name	Phonological stage	Plant part used	Reagent	Amount of HCN in ppm
1	*Celosia argentea* L.	Amaranthaceae	Kardu	Flowering	Leaves	C.C.	10
2	*Psoralea corylifolia* L.	Fabaceae	Bawachi	Flowering	Leaves	C.C.	75
3	*Terminalia bellirica* (Gaertn.) Roxb.	Combrataceae	Behda	Fruiting	Leaves Seeds	HCl HCl	20 00
4	*Terminalia catappa* L.	Combretaceae	Dashi Badam	Fruiting	Leaves Seeds	HCl HCl	00 10
5	*Tinospora cordifolia (*Wild) Miers.	Menispermaceae	Gulvel	Fruiting	Leaves Stem Fruit	C.C. C.C. C.C.	10 00 00
6	*Cajanus cajan* (L.) Millsp.	Fabaceae	Tur	Flowering	Leaves	C.E.	10
7	*Helianthus annuus* L.	Asteraceae	Suryaphul	Flowering	Leaves	C.E.	20
8	*Linum usitatissimum* L.	Linaceae	Jawas	Fruiting	Seed Fruit	HCl HCl	700 700
9	*Sorghum vulgare* Pers.	Poaceae	Jawar	Vegetative	Leaves (tiller) Seeds	HCl HCl	700 00
10	*Sorghum durra* (Forssk) Stap f.	Poaceae	Maldandi Jawar	Vegetative	Leaves	C.C.	180
11	*Sorghum dochna* (Forssk) Snowden	Poaceae	Dagadi Jawar	Vegetative	Leaves	C.C.	30
12	*Pennisetum americanum* (L.) K. Schum.	Poaceae	Bajari	Vegetative	Leaves	C.C.	500
13	*Zea mays* L	Poaceae	Maka	Fruiting	Leaves	C.C.	10

C.C. = Cuscuta caps, C.E. = Cuscuta extract

Results and Discussion

The results of detection and semi-quantitative estimation of HCN (Prussic acid) in fodder plants are presented in Table No.1. Out of the 13 fodder plants species tested, 12 were found positive for the presence of cyanogenic glycosides and HCN. The maximum amount of HCN is found to be 700 ppm in seeds and fruits of *Linum usitatissimum* L. and leaves of *Sorghum vulgare* Pers. In fact now it is a well established that the HCN production is a defence mechanism by plants but it is switched on only under particular circumstances.

The negative results are, however to be reconsidered as the HCN production by plants depends upon several internal and external factors as genes and environment (Jones, 1998) [6]. Cyanogenesis, however, cannot be considered as a chemotaxonamic parameter, but it is probably an interaction of plants against pests. The study could serve as reference to new studies regarding prussic acid in these plants.

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