breeding methods. In onion, pungency is an important property, as Indians like the pungent onions. Pungency in onion is positively correlated with better storage and good drying ratio[5].

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RNA AS A SUBSTRATE FOR THE ASSAY OF HEAT STABLE NUCLEASE OF *STAPHYLOCOCCUS AUREUS*

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THERMONUCLEASE serves as a good index of enterotoxigenic staphylococci in foods. This assay has been used in screening foods for the possible presence of enterotoxins[1–3], especially in situations where the number of cells was known to decline during processing and storage[4,5]. The agar well diffusion technique is widely used for thermonuclease assay[6]. Due to difficulties in procuring calf thymus DNA, which is also expensive, in the present work yeast RNA has been used as a suitable replacement, as it is easy to prepare and more economical.

Ribonucleic acid was purified from commercial yeast preparation according to Woodwards[7]. Enterotoxigenic strains of *Staphylococcus aureus* (FRI, S-100 and S-361) were procured from Dr M.S. Bergdoll, FRI, USA. Crude enzyme was prepared from cell-free supernatant of 24-hr-old culture in brain heart infusion broth impregnated with 5% skimmed milk powder after adjusting the pH to 3.8. It was steamed for 20 min, cooled and centrifuged for 30 min at 6000 rpm. The clear supernatant was kept chilled and used within 2 hr.

Ten different food materials such as raw beef, canned corned buff, canned meat-in-gravy, seasoned multipurpose food, plain multipurpose food, vegetable protein convalescent food, cooked rice, salted-dried mackerel, cake and frozen mutton mince were used for the nuclease assay. Twenty grams of food sample were spiked with 0.2 to 2 ml crude enzyme of *S. aureus*. Five grams of skimmed milk powder and 30–70 ml distilled water were added and the entire mass was blended. The homogenate was steamed for 20 min, cooled and centrifuged. The clear supernatant was used for the assay.

Gel diffusion agar assay[5] medium was employed with slight modifications. To 100 ml of acetate, tris and phosphate buffers of varying molarity and pH were added the following: agar 1%, purified yeast RNA 0.05–0.1%, calcium chloride 0.003 moles % and toluidine blue 0.0003 moles %. For slide assay, 2.5 ml of the compounded medium was pipetted on to 25 × 75 cm glass slide and chilled to set the medium. Wells (2 mm diam) were punched and equilibrated to 50°C. The wells were filled with 5 μl food extract and the slides were transferred to air-tight humid plastic boxes. For petri plate assay, 5.5 ml medium was poured into 13 × 50 mm petri plates and 5 mm wells were punched and 30 μl sample fluid was added. Slides as well as petri plates were incubated at two different temperatures (37 and 50°C) for 2, 4 and up to 18 hr.

The results with acetate, tris and phosphate buffers with different molarities and pH are depicted in table 1. Of the three buffers, phosphate buffer at 0.2 and 0.1 M at pH 7.0 incubated at 50°C for 2 hr has given optimum results. For consistent results 0.1 M phosphate buffer at pH 5.5 is recommended. Two hour incubation was considered optimal since no additional benefit resulted by extending the incubation up to 4 hr and beyond. This reduces the conventional DNase assay period by 2 hr[6,8]. Yeast RNA at 0.05% concentration was found to be optimal. Positive assay was obtained with ten different foodstuffs inoculated with both *S. aureus* strains.

Calf thymus DNA is an expensive material for the assay of Staphylococcal nuclease. Since *S. aureus* secretes nuclease which hydrolyses nucleotide polymers into 3′-nucleotides by breaking the phosphodiester linkages[9] and the organism is also known to excrete RNases[10], the present results with yeast RNA were found convenient and cheap substitute for calf thymus DNA. Further, the difficulty in discerning colour change with DNA medium leading to inconsistent results[3] could be avoided. Petri plate assay was more convenient than microslides, as larger (30 μl) volume of the food extract could be

Table 1 *RNase activity of S. aureus strains by slide assay**

	S – 100	S – 361
Acetate		
0.1 M pH 5.5	+	+
0.05 M pH 7.0	+	+
pH 9.5	+	+
Tris		
0.1 M pH 7.0	+	+
pH 9.5	+	+
0.2 M pH 7.0	++	++
pH 9.5	+	+
Phosphate		
0.02 M pH 5.5	+	+
pH 7.0	++	++
0.05 M pH 5.5	+	+
pH 7.0	++	++
0.1 M pH 5.5	+++	+++
pH 7.0	+++	+++
0.2 M pH 5.5	+++	+++
pH 7.0	++	++

*Ratings are based on the brightness and diameter of the pink zone; +, < 5 mm diameter; ++, 6–8 mm diameter; +++, > 8 mm diameter.

used in the test. RNA assay medium can also be stored at ambient temperature for over two months as DNA assay medium. The most important advantage is the clarity of the pink colour change in the blue background of the assay medium with RNA as substrate.

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EFFECT OF FUNGICIDE ZINEB ON THE LEACHING OF MICRONUTRIENTS FROM THE LEAVES OF *ZEA MAYS* L.

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PESTICIDAL spray is known to alter the leaf leachate constitutents, which in turn have a direct effect on the disease incidence[1]. Leaching of substances has also been implicated in the yield, quality and nutritive value of economic food plants[2] and it may also intensify in certain physiological disorders[3]. We had earlier reported[4] some of the microbiological changes in *Cicer arietinum* due to pesticidal sprays. An attempt has been made in the present study to see the effect of zineb on the leaching of micronutrients from leaves.

Zea mays L. grown under field conditions was sprayed with zineb, when the crop was 40-day-old. One plot left unsprayed as control. Leaf samples from the two plots were collected on 1st, 3rd, 5th, 10th, 15th, 25th and 40th day during post-spray period. Leaf leachates of control and pesticide-treated leaves were collected as recommended by Godfrey[5] in water by immersing 10 g of freshly collected leaves in 100 ml of sterile glass distilled water for 6 hr. Free iron content in the leaf leachates was estimated employing the technique recommended by Ryan and Botham[6] using 1–10 phenanthroline reagent. Sodium and potassium were estimated using the flame photometer (Elico, Hyderabad model CL-22,) and the concentrations read from standard graphs made earlier using sodium chloride and potassium chloride respectively.

Table 1 shows that amount of micronutrients in the leaf leachates of control and sprayed leaves. There is increase in the content of iron and potassium in sprayed samples. There is not much