Summary
1. This study critically examined the morphology, histochemistry, protein profile and spectroscopic analysis of chorion or eggshell of two Indian paddy field grasshoppers *Oxya hyla hyla* and *Gesonula punctifrons*.

2. Chorion started to form beneath the follicular cell layer from the early stage of development i.e. follicular cell stage. Chorion secretion attained its optimum level when follicular cells were in their highest maturity and had two to three cell layer thicknesses.

3. In *O. hyla hyla* follicle stage egg, the thickness of the chorionic layer varied approximately from 14.3µm to 73.7µm and chorion had highest thickness at the terminal end of posterior pole and lowest at the anterior pole.

4. In *G. punctifrons* follicle stage egg, thickness of chorion ranged from 23.7 µm to 45.4 µm. Highest thickness was found at both sides of posterior pole but not in the terminal pole region.

5. Thickness of *Oxya* oviduct stage egg chorion ranged from 14.2µm to 137 µm. The highest thickness of the chorion layer was observed in the posterior pole. Lowest thickness was found in the anterior pole.

6. In *Gesonula* oviduct stage egg, the thickness of chorionic layer varied from 25.1 µm to 234 µm. The thickness of the chorion was highest in the posterior pole and lowest in the anterior pole.
7. *O. hyla hyla* immature oocyte had single follicle cell layer. Shapes of the follicle cells were almost round. Follicle cells were slightly PAS positive, faintly pink in colour. Initiation of chorionic secretion was observed.

8. In *G. punctifrons* the immature oocyte was almost PAS negative showing a faint pink colour. Single follicle cell layer was present. Follicle cells were almost round in shape. Chorionic secretion was observed from some of the follicle cells.

9. In matured follicular stage egg of *O. hyla hyla*, follicle cell layer had 2 to 3 layers of cells. Follicle cells became elongated in shape. The chorionic secretion had PAS positive reaction and was pink in colour.

10. In case of more eggs matured follicle cell layer had 2 to 3 cell layers. Follicle cells were elongated in shape and were less thick. The chorionic secretion took pink colour in PAS reaction.

11. Immature egg of *O. hyla hyla* had single cell layer of follicle cells in which little chorionic secretion was observed. Chorionic secretion took blue colour in Mercurry- bromphenol blue staining.

12. In *G. punctifrons* immature egg, two layers of follicle cell were found. Here minimum amount of secretion of chorionic layer was found which took blue colour in Mercurry- bromphenol blue staining.
13. The findings suggested that chorion had good amount of sugar moiety and proteinaceous material.

14. SEM studies revealed that follicle stage egg of *Oxya hyla hyla* was approximately 3.166mm thick. Whole surface of the eggshell was studded with minute spicules. Some spicules with minute straight numerous foldings started to form a ‘cap’ like structure.

15. Follicle stage egg of *Gesonula punctifrons* was approximately 2909µm. The chorion had some small spicules like structures and some circular foldings throughout the egg surface.

16. Length of oviduct stage egg of *Oxya hyla hyla* was approximately 3.22mm. Groove like foldings were present in the middle zone of the egg. In the posterior pole the ‘cap’ like structure was present. Micropylar zone contained micropylar pores scatterly present on it.

17. In oviduct stage eggshell of *Gesonula punctifrons*, the surface area of the chorion was plain without any sculpture. The egg was approximately 3938 µm in length. Less developed ‘cap’ like architecture was present in this stage.

18. Length of after laying stage egg of *Oxya hyla hyla* was approximately 3.277mm. The whole surface of the eggshell was covered by pentagonal and hexagonal ridges. The ‘cap’ in the posterior zone was fully formed in this stage.
19. The egg surface of *G. punctifrons* showed a sculptured structure in after laying stage. The whole length of the egg was approximately 4286 µm. A ‘cap’ like structure was also present at the posterior pole of the egg. Micropylar region was present at the top of the anterior pole.

20. Structural features were lowest in follicular cell stage and structural modifications started to begin from this stage.

21. Micropylar pores may facilitate the sperm entry in the oocyte which allows fertilization. At the posterior end of the chorion in both insects ‘Cap’ like structure started to form from follicular cell stage and found in every stage of development which might facilitate hatching.

22. It has been shown by TEM studies that in the follicle stage eggshell of *G. punctifrons*, five distinct layers were present. These were vitelline membrane, innermost chorionic layer, air layer and the outer chorionic layer. Follicle cell layer was also present.

23. In *G. punctifrons* oviduct stage egg, four layers were present, the vitelline membrane, innermost chorionic layer, air layer and the outer chorionic layer. Thread like proteinaceous structures and polysaccharides droplets were present.
24. After laying stage eggshell had four layers, the vitelline membrane which was little bit stretched, innermost chorionic layer, air layer and the outer chorionic layer. Proteinaceous structures and polysaccharides droplets were present in much condensed manner.

25. Follicle stage egg of *O. hyla hyla* had four distinct layers: vitelline membrane, interchorionic layer, air layer and fully formed chorionic layer.

26. In oviduct stage egg of *O. hyla hylai*, four layers of eggshell were present. The vitelline membrane, inner most chorionic layer, air layer and outer chorionic layer. Droplets of polysaccharides were also present with protein deposition as thread like structures.

27. In after laying stage four chorionic layers were present: the vitelline membrane, innermost chorionic layer, outer chorionic layer and air layer. Condensation of the proteinaceous structures and polysaccharide droplets were observed.

28. Unique solvent for highest solubilization of chorion was developed containing 6M Urea, 4% β-Mercaptoethanol, 400mM Tris-HCl (pH 8.4), 1% SDS and boiling that in water bath for 5 to 7 minutes.

29. In *Gesonula* follicle cell stage, oviduct stage and after laying stage had 17, 7 and 6 polypeptides respectively.
30. In *Oxya* follicle cell stage, oviduct stage and after laying stage had 8, 12 and 6 polypeptides respectively.

31. 55kDa protein was found in both insects in first two developing stages of the egg.

32. Calcium, Zinc, Iron and Copper were involved in chorion hardening. Amount of these components was increased with advancement of stage of maturation.

33. Tyrosine and Tryptophan were present in chorion in high concentration.

34. Mass spectral sequence analysis revealed that the 55kDa protein had different amino acid sequence in *Oxya* and *Gesonula*.

35. Structural analysis through computational method revealed that in *Oxya*, the 55kDa protein had structurally closest identity with envelope glycoprotein of HIV 1. Presence of 39 groups, 305 atoms and 309 bonds were revealed in this protein.

36. In *Gesonula*, the 55kDa protein has identity with ATPase. Computational analysis revealed presence of 28 groups, 216 atoms and 220 bonds in this protein.