International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Int. J. of Life Sciences, 2021; 9 (2):205-210
ISSN:2320-7817(p) | 2320-964X(o)

Research Article Open Access

Effect of Azadirachtin on the haemolymph protein profile of lepidopteran pest *Pericallia ricini* (Lepidoptera: Arctiidae)

Gnanamani R[1*] and Ramanathan B[2]

[1]Assistant Professor, PG and research Department of Zoology, Yadava College, Madurai, Tamilnadu, India.
*Corresponding author Email: gnanamani87@gmail.com

Manuscript details:

Received: 10.04.2020
Accepted: 26.06.2021
Published: 30.06.2021

Cite this article as:
Gnanamani R and Ramanathan B[2] (2021) Effect of Azadiractin on the haemolymph protein profile of lepidopteran pest *Pericallia ricini* (Lepidoptera: Arctiidae), India, *Int. J. of Life Sciences*, 9 (2): 205-210.

Available online on http://www.ijlsci.in
ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

ABSTRACT

SDS-PAGE analyses are important for better understanding of insect development and physiological process. There is a significant change has been observed in haemolymph protein concentration and protein bands and its staining intensities. SDS-PAGE protein profile of haemolymph of control revealed the presence of about 18 stained polypeptide bands of varying intensities with molecular weight ranging from 14 to 97.4kDa. The protein content was found decreasing and increasing with the increased concentration of Azadirachtin treatment but the untreated set recorded maximum value. The staining intensity of 45kDa protein band was observed as a major band and also15kDa, 20kDa, 25kDa, 32kDa and 35kDa protein fraction has been observed as a thick band in the treatment. Consequently, there was a significant decrease of protein concentration was noticed with respect to subsequent treatments based on the appearance, disappearance and staining intensity of the protein bands.

Keywords: Azadirachtin, *Pericallia ricini*, SDS-PAGE

INTRODUCTION

Studies of protein patterns are necessary for better understanding of the insect development, as proteins are the first conceivable product of gene activity. Toxicity response in most organisms was mostly all the way through the mobilization of tissue proteins (Saleem and Shakoori, 1986; Saleem *et al*., 1998). Proteins were produced or conked out in retort to different dosage of diverse pesticides. During the final larval instar of holometabolous insects, these proteins virtually accounted for 70 - 80% of the total soluble proteins by weight (Kanost *et al*., 1990; Telfer and Kunkel, 1991).

Haemolymph of insect larva contained several high molecular weight proteins like arylphorin (Telfer and Kunkel, 1991) and lipophorins (Shapiro *et al*., 1988) in addition to the storage proteins. All the proteins

were synthesized in the fat body and liberated into the haemolymph (Brookes, 1969; Pan *et al*., 1969; Hegedorn *et al*., 1973; Gelti - Douka *et al*., 1974; Kunkel and Lawler, 1974; Chen *et al*., 1976; Koeppe and Oftengand, 1976) and it has to be integrated later on into different organs including ovaries (Rohrkasten and Ferenz, 1985, Nordin *et al*., 1990 and Valle, 1993). The foremost haemolymph proteins which had been described so far included storage proteins, lipoproteins, ovarian proteins, metal binding proteins and hormone carrier proteins (Kanost *et al*., 1990). Investigation of haemolymph proteins as a result of SDS-PAGE and densitometry showed that the quantity of haemolymph proteins were diminished considerably in the parasitised larvae of *P. ricini* (Fabricius) (Raja *et al*., 2000).

Pericallia ricini is found all over India and is commonly known as castor hairy caterpillar. The black hairy caterpillar is a gluttonous leaf eater of a mixture of vegetables which is one of the most important vegetable crops of Tamilnadu (David and Ananthakrishnan, 2004; Vanitha *et al*., 2011). The female moths lay eggs in large number on the lower surface of leaf. The larvae feed on young and full grown plant leaves and fruits. In heavy infestation only stem and branches are left behind (Nathan and Nathan, 2011). When the larval population was controlled, the crop yield may extend up to 70 percent to 80 percent. (Pandey *et al*., 1981).

The well known nature product, Azadirachtin from the neem tree *Azadirachta indica*, causes an anti- ecdyson effect and hampered with insect ecdysis (Sieber and Rembold, 1983). It is a powerful insect anti-feedant and repellents (Butterworth and Morgan, 1971). It might also interrupt the growth; restrain moulting (Koul *et al*., 1987) and oogenesis (Naumann and Isman, 1995). It could be eco-friendly, selective, non-mutagenic with little noxious to mammals (Mordue and Nisbet, 2000). Neem seed kernel extracts were found to act both as ovipositional deterrent and repellent to insects including mosquitoes (Dimetry *et al*., 1995; Chen *et al*., 1996). The neem tree *Azadirachta indica* A.Juss has shown hopeful results for the control of insects, as well as the important pests of agriculture are vulnerable to diverse behavioral and physiological effects of neem (Schmutter, 1990). Since, information regarding the effect of Azadirachtin on Hemolymph proteins is scanty; an attempt has been made in the present investigation to study the effect of Azadirachtin on the protein profile in haemolymph of polyphagous pest *P. ricini*

MATERIAL AND METHODS

Culture the larvae in the laboratory

Freshly emerged first instar larvae of *P.ricini* were collected from the castor plant and separated. These larvae were maintained in the lab condition for the experimental purpose.

Azadirachtin treatment

Azadirachtin 0.5% (Neemazal F) was used for the assays. Azadirachtin was diluted in acetone and different concentrations were prepared. It was orally administered into the larvae. Newly emerged fifth instar larvae of *P.ricini* were introduced in separate containers. For oral treatment, Fresh castor leaves were immersed in the 50ppm, 75ppm and 100ppm Azadirachtin solution (control leaves in distilled water) for 30 seconds drained and allowed to dry for 30minutes on a filter paper. Twenty larvae per concentration were used for all the experiments. And this experimentation was repeated three times at different time intervals such as 24hrs and 48hrs.

Collection of haemolymph

For haemolymph collection, the insects were frozen for 15 min at 4 ^{0}C. it was cleaned with 70% (v/v) ethanol solution. Haemolymph samples were attaining by pricked the larval abdomen with a sterilized needle. Haemolymph was directly poured into germ-free and frozen Eppendorf tubes which have a few crystals of phenylthiourea (PTU) to avoid melanization. The hemocyte-free haemolymph was obtained by centrifugation at 200g for 5 min. Then the supernatant was spun down at 20,000g for 15 min at 4^{0}C to pellet cell debris (Hyrsl and Simek, 2005). Molecular weight determination of total proteins was done in haemolymph by using the SDS - PAGE as described by Laemmli (1970)

RESULT AND DISCUSSION

SDS-PAGE are employed in the study to analyze the specific fine details of haemolymph protein profile as

well as to identify proteins formed in response to plant chemical such as Azadirachtin and pesticidal challenge in the larval developmental stage. The electrophoretic outline of haemolymph protein of both untreated and treated larval insects has revealed the different pattern or developmental stages. Studying protein profile of an insect is of considerable importance for understanding different physiological processes. The separation and characterization of the single proteins facilitates the study of the chemical nature and physiological function of each protein (Mohamed, 1990). SDS-PAGE protein profile of haemolymph of control revealed the presence of about 18 stained polypeptide bands of varying intensities with molecular weight ranging from 14 to 97.4kDa (Fig.1).

The protein bands are of two categories via major, minor based on their staining intensities. The protein content was found decreasing and increasing with the increased concentration of Azadirachtin treatment but the untreated set recorded maximum value. Polymorphic variations were noticed with regard to the number of protein bands in the haemolymph during the fifth instar development with different treatments. SDS-PAGE profile of haemolymph protein showed perceptible disparity in several major proteins in control and treated insects. Even though, some protein is present in both the control and treated insects but intensity of these proteins were varied.

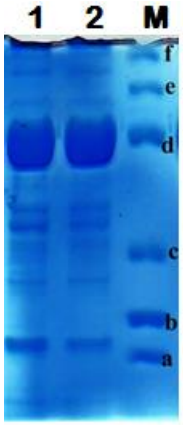

Figure 1. SDS- PAGE protein profile of haemolymph proteins of untreated last instar *P. ricini*.
1.24hrs Azadirachtin control; 2- 48hrs Azadirachtin control; M- a- 14.3 KDa, b- 20.1KDa, c- 29KDa, d- 43KDa, e- 66KDa, f- 97.4KDa

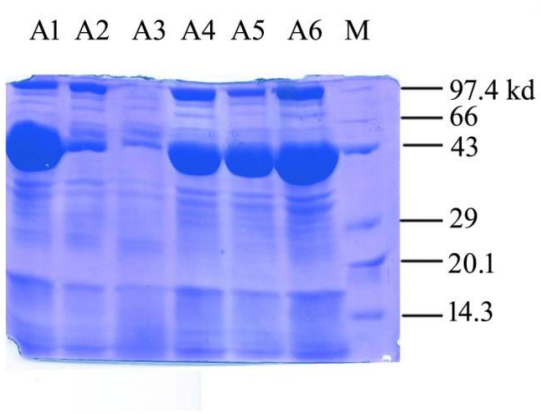

Figure 2. SDS- PAGE lane profile graph of haemolymph proteins of last instar *P. ricini* treated with Azadiractin oral administration.
A1-24hrs Azadiractin oral administration at 100ppm; A2-48hrs Azadiractin oral administration at 100ppm; A3-24hrs Azadiractin oral administration at 75ppm; A4-48hrs Azadiractin oral administration at 75ppm; A5-24hrs Azadiractin oral administration at 50ppm; A6-48hrs Azadiractin oral administration at 50ppm; M-Marker

Wyatt and Pan (1978) noted the entire number of protein bands of haemolymph varies from 10-30 in varies cluster of insects. Accordingly in *P. ricini* the presence of about 18 stained polypeptide bands of varying intensities with molecular weight ranging from 14 to 97.4 has been observed. 45KDa and 97kDa protein bands were observed as a major protein in control as well as treated insects. Larval hemolymph protein profiles emphasized a dominant group of protein bands ranging in molecular weight from 66 to 97kDa. These are considered to be storage proteins according to Miller and Silhacek (1982) and Godlewski et al. (2001). Control insects appeared to contain large amount of storage proteins. Storage proteins are very well known as an amino acid reserve for the production of mature proteins. This suggested that these proteins are produced in the fat body of final instar and entered into haemolymph (Kunkel and Nordin, 1985; Rohrkasten and Ferenz, 1985).

The Electrophorectic patterns and Densitometric analysis of the Azadirachtin oral application of treated insects are presented in Fig.2. It showed variation in the number and prominence of bands of Azadirachtin treated compared to those of control insects. The protein content was higher in the initial concentration and decreased at the 75ppm and significantly increased at the final concentration. Many extra bands were observed in all the concentration. There was appearance of new protein fraction has been noticed between 14kDa and 45kDa respectively. The intensity of 45kDa protein fraction was decreased in 75ppm concentration of 24hrs treatment and also 97kDa protein fraction was appeared in trace amount. The intensity of the 45kDa protein fraction was decreased in the 100ppm concentration of Azadirachtin when compared with the control and other treatment. One 45kDa protein part in larval haemolymph was notably more rich in larvae of *P. ricini* .The molecular weight of this protein was similar in size to Cytochrome P450 enzymes reported for other insects (Scharf et al., 2000). The Cytochrome P450 monooxygenases are significant metabolic system included in the detoxification of xenobiotics and insecticide resistance (Berge et al., 1998; Brown et al., 2003). The P450 enzymes are also vital for the adaptative mechanisms of insects to the toxic chemicals produced by their host plants (Gould, 1984). Hence, suggested that presence of 45KDa protein bands may be a outcome of metabolic resistance reaction to Plant chemicals or its oxidatively activated products, because substrates for P450 include insecticides, allelochemicals, hydrocarbons and drugs (Halliwell et al., 1999).

It is worth mentioning to note 15kDa, 20kDa, 25kDa, 32kDa and 35kDa protein fraction has been observed as a thick band in the treatment. But it was found to be as a trace amount in 75ppm treatment. Consequently, there was a significant decrease of protein concentration was noticed with respect to subsequent treatments based on the appearance, disappearance and staining intensity of the protein bands. In the present study the quantitative increase or decrease in common protein bands among the tested treatments may be due to the presence of different numbers of iso-genes responsible for the production of storage protein type or due to the prolongation of the genes related to this protein in their action compared to the other treatments. The inhibition of some protein bands can explain some other experimental results such as reduction in weight, slow development, tissue degeneration (Schloter, 1985). Rao et al. (1999) and Ayeed et al. (2001) revealed that the protein pattern differences may act as a tool to identify (estimate) the similarity and genetic distance between the control and treated samples. Alteration in protein profile of various tissues were experiential by numerous investigators (Koopmanschap et al., 1992; Damara and Gupta, 2010; An and Kanost, 2010) and the effect of hormone administration on larval-pupal development in sesricigenous insect is reported well (Kumar et al., 2008; Nair et al., 2008). It is also accounted that proteins are most important substances that are efficient in forming complexes with the divalent metals in the haemolymph of insects (Florkin and Jeuniaux, 1974; Mullin, 1995; Choudhury et al., 2004; Kumar et al., 2008; Damara and Gupta, 2010; An and Kanost, 2010).

Studies on protein profile of fifth instar of *P.ricini* especially exposed to different treatments with plant chemical Azadirachtin have been highlighted in this work. Interestingly, the effect of plant chemicals on insects has shown that haemolymph proteins might be reduced due to antifeedant activity (Padmaja and Rao, 2000). Hence, the disappearance of few bands in the treated insects, fall in line with above reports. Similar observations has been found in Azadirachtin treated *S. litura* (Ayyangar and Rao, 1990; Neoliya et al., 2007). The probable cause of haemolymph proteins decline may be due to the influence of plant products on endocrine glands of insects, which results in the changes of spectrum of body proteins.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

An C, Kanost MR (2010) *Manduca sexta* serpin-5 regulates prophenoloxidase activities and the toll signaling pathway by inhibiting haemolymph proteinase HP6. *Insect Biochem. Mol. Biol.* 40(9): 683–689. doi: 10.1016/j.ibmb.2010.07.001

Ayeed TH, Dorrah MA, Shaurub EH, EI-Sadawy HA (2001) Effect of the entomopathogenic nematode, *Heterohaboditis bacteriophora* HPSS and Azadirachtin on the immune defense response and prophenoloxidase of *Parasarcophaga larva* (Diptera: Sarrcophagidae). *J. Egypt. Soc.Oarasitol*. 31(1), 295-325.

Ayyangar GSG, Rao PJ (1990) Changes in haemolymph constituents of *Spodoptera litura* (Fabr) under the influence of Azadirachtin. *Indian. J. Ent*. 52(1), 69-83.

Berge JB, Feyeresien R, Amichot M (1998) Cytochrome P450 monooxygenase and insecticide resistance in insects. Phil. Trans. R. Soc. Lond.B. 353, 1701-1705.

Brookes VJ (1969) The induction of Yolk synthesis in the fat body of an insect Leucophase moderate by an analogue of the juvenile hormone. Dev.Biol. 20, 459-471.

Brown D, Zhang L, Wen Z, Scott JG (2003) Induction of P450 monooxygenase in the German Cockroach, *Blattela germanica*,L.Arch.Insect. Physiology. 17, 969-977.

Chen CC, Dong YJ, Chen LL, Hou RF (1996) Deterent effect of neem seed kernel extract on oviposition of the oriental fruit fly (Diptera: Tehritidae) in guava. J. Econ. Entomon. 89, 462-466.

Chen TT, Couble P, De Luca FL, Waytt GB (1976) Juvenile hormone control of vitellogenin synthesis in *Locusta migratonia* in the juvenile hormones. L.I. Gilbert. (Editor). Newyork Plenum. 505-529.

Chodhury A, Saikila JR, Unni BG (2000) Studies on certain enzymes and metabolites of carbohydrates metabolism and changes in haemolymph protein profile during development in the silkworm, *Antheraea assama* and *Philosamia ricini*. Asian journal of Microbiol. Biotech. Environ.Sci. 6, 13-20.

Damara M, Gupta AD (2010) Identification of 86kDa protein as methionine rich hexamenin in the rice moth, *Corcyrs cephalanica*. Comparative Biochem.Physiol. part B: Biochem. Mol.Biol. 157, 229-237.

Dimetry NZ, Barakat AA, Abdalla EF, Elmetwaly HE, Abd El-Salam AME (1995) Evaluation of two neem seed kernel extracts against *Liriomyza trifoli* Burg (Diptera: Agromyzidae). Anz Schalingskde Pflanzenschutz Umweltschut, 68, 39-41.

Florkin M, Jeunieux C (1974) Haemolymph comparison. In: Physiology of Insects. Rockstein, M. (Ed). Academic press, Newyork. 5(2), 255-307.

Gelti-Douka H, Gingeras TR, Kambysellis MP (1974) Yolk proteins in Drosophilla identification and site of synthesis. J.Exp.Zool. 187, 167-172.

Godlewski J, Kludkiewicz B, Grzelak K, Cymborowski B (2001) Expression of larval haemolymph proteins (Lhp) genes and protein synthesis in the fat body of greater wax moth (*Galleria mellonella*) Larvae during diapauses. J. Insect. Physiol. 47, 759-766.

Gould F (1984) Mixed fuction oxidase and herbivore polyphagy. Ecol. Entomol. 9, 29-34.

Halliwell B, Gutteridge JMC (1999) Free radical in biology and medicine. Third ed. Oxford university press, Oxford.

Hegedom HII, Fallon AM, Laufer H (1973) Vitollogenin synthesis by the fat body of the mosquito *Ades aegypti*: evidence for transcriptional control. Dev. Biol. 31, 285-294.

Kanost MR, Dawooga JK, Ryan RO, Husden MD, Zeilger R (1990) Insect Haemolymph proteins in advance. Insect Physiology. 22, 299-397.

Koeppe J, Oftengand J (1976) Juvenile hormone induced biosynthesis of vitellogenin in *Leucophaaea maderae*. Arch. Biochem. Biophy. 173, 100-113.

Koopmanschap B, Lammers H, Kort S (1992) Storage proteins are present in the haemolymph from larvae and adults of the Colorado potato beetle. Arch. Insect. Biochem. Physiol. 20, 119-133.

Koul O, Amanai K, Ohtaki T (1987) Effect of Azadirachtin on the endocrine events of *Bombyx mori*. J.Insect. Physiol. 33, 103-108.

Kumar D, Subramanyam B, Sharan SK, Mishra PK, Singh BMK, Suryanarayanan N (2008) Endogenous 20-hydroxyl ecdyson level in haemolymph of non diapauses- destined and diapauses destined generations of tasar silkworm, *Antheraea mylita* Drury and associated development changes. Eur.J.Entomol. 105, 591-508.

Kunkel JG, Lawler DM (1974) Larval specific serum proteins in the order Dictyoptera and Immunological characterization in larval *Blattella gerinanica* and cross reaction throughout the order. Comp.Bochem.Physiol. 47, 697-710.

Junkel JG, Nordin JH (1985) Yolk proteins. In Kerkut G.A and Gilbert, L.I (Eds): Comprehensive Insect Physiology, Biochemistry and pharmacology. 1, 84-11.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227, 680-685.

Miller SG, Silhacek DL (1982) Identification and purification of storage proteins in tissues of the greater wax moth *Galleria mellonella* (L). Insect. Biochem. 12, 277-292.

Mohamed MI (1990) Sterility and some associated physiological changes in the adult cowpea weevil, *Callosobruchus maculates* (F). Ph.D. Thesis, Dept. Entomol. Fac. Sci. Ain Shams Univ.

Mordue (Luntz) AJ, Nisbet M (2000) An Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. An. Soc. Entomol. Brasil. 29(4), 615-632.

Mulin DE (1995) Chemistry and physiology of the haemolymph in: Comprehensive Insect Physiology and pharmacology, Kerkut, G.A and L.I. Gilbert (eds). Pergamon press, Oxford. 355-400.

Nair KS, Kariappa BK, Kamble CK (2008) Impact of individual and co administrations of juvenile hormone analogue and phytoecdysteroid on the crop management and performance of silkworm, *Bombyx mori*. L. J. Biol.Sci. 8, 470-473.

Nathan LS, Nathan RJ (2011) Effect of chitin Biosynthesis Inhibitor Diflubenzuron on the Fifth instar larvae of *Pericallia ricini* fabr. The Asian journal of animal science. 6(!), 66-70.

Naumann K, Isman MB (1995)Evaluation of neem Azadirchta indica seed extract and oil as oviposition deterrents of noctuid moths. *Entomol. Ex. Appl.* 76, 115-120.

Neoliya NK, Dwijendra S, Sangawan RS (2007) Azadirachtin based insecticides induce alternation in *Helicoverpa armigera* Hub. Head Polypeptides. *Current Science*. 92(1), 94-99.

Nordin JH, Beaudoin EL, Liv X (1990) Proteolytic processing of *Blateliager flafica* vitellin during early embryo development. *Arch. Insect. Biochem.Physiol*. 15, 119-135.

Padmaja PG, Rao PJ (2000) Effect of plant oils on the haemolymph proteins of final instar larvae of *Helicoverpa armigera* Hubner, *Entomon.* 25(2), 107-115.

Pan ML, Bell WJ, Telfer WH (1969) Vitellogenic blood protein synthesis by insect fat body. *Science.* 165, 393-394.

Pandey GP, Doharey RB, Varma BK (1981) Efficacy of some vegetable oils for protecting green gram against the attack of *Callosobruchus maculates*(Fabr). *Indian J. Agri.Sci*. 51, 910-912.

Raja N, Janhanan S, Ignacimuthu S (2000) Changes of haemolymph protein profile in the larvae of *Pericallia ricini* (Fab) paratisied by the braconid wasp, *Apanteles taragarnae* Viereck (Hymenoptera: Braconidae). *Indian J.Egypt. Biol.* 38(4), 393-395.

Rao PJ, Kumar KM, Singh S, Subramanyam B (1999) Effcet of Artemisia annua oil on development and reproduction of *Dysdercus koenigii* F. (Hem: Pyrrhocoridae). *J. Applied Entomol*. 123(5), 315-318.

Rohrkasten A, Ferenz HJ (1985) Invitro study of selective endocytosis of vitellogenin of locust oocytes. Roux's. Arch. Dev. Biol. 194, 411-416.

Saleem MA, Shakoori AR (1986) Biochemical effects of sublethal doses of cypermethrin on the sixth instar larvae of *Tribolium castaneum* (Herbst). Archives of Insect Biochemistry and Physiology. 3(5), 447-455.

Saleem MA, Shakoori AR, Mantle D (1998) Macromolecular and enzymatic abnormalities induced by a synthetic pyrethroid cypermethrin in adult beetles of a stored grain pest, *Tribolium casteneum* (Herbst Coleoptera: Tenebrionidae). Archives of Insect Biochemistry and Physiology. 39(4), 144-154.

Scharf ME, Siegfried BD, Meinke LJ, Wright RJ, Chandler LD, (2000) Cytochrome P450 mediated N- demethylation activity and induction in insecticide resistant and susceptible western corn rootworm populations (Coleoptera: Chrysomelidae). Pestic. Biochem. Physiol. 67, 137-143.

Schloter H (1985) Occurrence of weight gain reduction and inhibition of metamorphosis and storage protein formation in last larval instars of Mexican bean beetles, *Epilachna varivestis* after injection of Azadirachtin. Entomol. Exp. Appl. 39(2), 191-195.

Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree *Azadirachta indica*. Annu. Rev. entomol. 35, 271-297.

Shaprio JP, Law JH, Wells MA (1988) Lipid transport in insects. Annu. Rev. entomol. 29, 297-318.

Sieber KP, Rembold H (1983) The effects of Azadirachtin on the endocrine control of molting in *Locustus migratoria*. J.Insect. physiol. 29,523-528.

Telfer WH, Kunkel JG (1991) The function and evolution of insect storage hexamers. A. Rev Entomol. 36, 205-228.

Valle D (1993) Vitellogenesis in insects and other groups. A review, Mem. Inst. Oswaldo. Cruz. 88, 1-26.

Vanitha V, Thiyagesan K, Baskaran N (2011) Prevalence of intestinal parasites among captive Asian Elephants *Elephas maximus*. Effect of season, host demography and management systems in Tamilnadu. Journal of Threatened Taxa. 3(2), 1527- 1534.

Wyatt GR, Pan ML (1978) Insect plasma proteins. Ann. Rev. Biochem. 47, 779-817.