DISCUSSION

The results of present investigation on the biopesticidal effect of *Jatropha curcas* seed, oil and meal on the four pests, *Helicoverpa armigera*, *Bemisia tabaci*, *Cnaphalocrosis medinalis* and *Diacrisia obliqua* and biochemical investigations of its effect on activities of three enzymes amylase, protease and lactate dehydrogenase in third instar larva of *Helicoverpa armigera* have been discussed in this chapter under various heads.

*Jatropha curcas*, in India is presently being cultivated as a biodiesel plant with 35–40% of oil depending on the variety. Gubitz *et al.* (1999) have reported that the fatty acid composition of *Jatropha* oil is similar to that of oil used for human consumption. A total of 30–32% of crude protein can be obtained as a meal (Makkar, *et al.*, 1997). Apart from being a source of oil and the meal being highly nutritious, it can be used as an animal feed (Aregheore, *et al.*, 2003). However, despite their advantage, the seeds are reported to contain antinutritional factors such as saponin, phytate, trypsin inhibitor, glucosinolates, amylase inhibitors, and cyanogenic glucosides (Rakshit, *et al.*, 2006, 2008). In addition to above, toxic/irritant compounds have been identified in the seed which include, β-D-glycosides of sitosterol (Bose, *et al.*, 1961), curcin (Stripe *et al.*, 1976), flavonoids, vitexine and isovitexine (Sankara, *et al.*, 1971) and 12-deoxy 16-hydroxy phorbol (Adolf *et al.*, 1984). The whole seed as well as seed meal are reported to be highly toxic in animal studies (Adam and Magzoub, 1975). The seeds of *J. curcas* are known to be toxic in mice and rats (Adam, 1974; Stripe *et al.*, 1976).
Adam and Magzoub (1975) have reported that the Nubian goats fed with *J. curcas* seeds at doses ranging from 0.25 to 10 g/kg/day, were found to be toxic with mortality occurring between 2 and 21 days. Accidental ingestion of *Jatropha* seeds by children aged 3–5 years led to restlessness, severe vomiting and dehydration (Levis, *et al*., 2000).

The experiments conducted by Solsoloy (1995), showed the insecticidal potential of the crude oil extracted from *J. curcas* on cotton bollworm and flower weevils. In addition, it was also reported to have controlled stored grain pests in corn, rice and mungbean. On the other hand, golden snail, a major rice pest could also be controlled through dust formulation. Likewise, it is anticipated that with the proper formulation, it wards off household pests such as cockroaches, rats and houseflies.

Snails act as intermediate hosts of schistosomes in many tropical countries. A study by Liu, *et al.* (1997) reported molluscicidal activity of *Jatropha* extracts containing phorbol esters against *Biomphalaria glabrata*, *Bulinus globosus*, and *Oncomelania hupensis*.

Extracts from *J. curcas* L. were also found to be toxic against snails transmitting *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium*, respectively (Liu *et al.* 1997; Rug and Ruppel, 2000).

Insecticidal activities of *Jatropha* oil containing phorbol esters have been reported in *Manduca sexta, Helicoverpa armigera, Aphis gossyii, Pectinophora gossypiella, Empoasca biguttula, Callosobruchus chinensis, Sitophilus zeamays, Phthorimaea operculella, Culex sp., Sesamia calamistis,*
Busseola fusca, Periplaneta Americana, Blatella germanica, and Oncopeltus fasciatus (Wink et al. 1997).

The seeds of *J. curcas* have been shown to have nematicidal, fungicidal (Sharma and Trivedi, 2002), antifeedant (Meshram, et al., 1996), molluscicidal (Liu, et al., 1997) and abortifacient (Goonasekara, et al., 1995) activities.

Based on the above reports, *Jatropha curcas* was selected as a plant of choice for this investigation as a botanical pesticide. A trial has been made to analyze the effect of its seed, meal and oil in controlling different pests attacking different crops.

Based on the chemical nature and solubility of the major antinutritional factors present in the seed, following are the most commonly used solvents selected for the preparation of their extracts. They are methanol, hexane, ethanol, petroleum ether, ethylacetate, benzene and water (referred here and after as aqueous extract).

Based on the chemical nature and solubility of the anti-nutritional factors present in the *Jatropha curcas* seed, different solvents were selected for the preparation of extracts of *Jatropha curcas* seed, oil and meal.

One of the major antinutritional factors present in the meal is phorbol esters which are mostly insoluble in water and soluble in most organic solvents. The structure of the phorbol esters is dependent on the tetracyclic diterpene carbon skeleton known as tigliane. Tigliane is the fundamental
alcohol moiety in the phorbol esters. Tigiane contains four rings designated as A, B, C and D (Fig.5.1.) Hydroxylation of this basic structure at different positions and then ester bonding to various acid moieties results in formation of large varieties of phorbol ester compounds. The phorbol, the parent diterpene of phorbol esters, contains five hydroxyl groups. This chemical nature of the phorbol esters makes them to be moderately polar, and ethanol and methanol have major affinity for them. The use of methanol and ethanol solvents has the advantage of it.

**STRUCTURE OF PHORBOL ESTERS**

![Chemical structures of phorbols](image)

**Fig.5.1. Chemical structures of phorbols**
Saponins comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone (sapogenin) linked to one or more oligosaccharide moieties by glycosidic linkage. The carbohydrate moiety consists of pentoses, hexoses, or uronic acids. They have both polar (sugar) and nonpolar (steroid or triterpene) groups. Specifically, they are amphipathic glycosides.

Lectin is generally considered to be another toxic factor in *J. curcas* seeds (Panigrahi, *et al.*, 1984). The lectin (curcin) of *J. curcas* seeds has been reported to be much less toxic than the well-known lectins, ricin and abrin (Makkar, *et al.*, 1997). But it is unclear which curcin has biological and enzymatic activities (Lin, *et al.*, 2003). Curcin is a toxalbumin. Higher agglutination, observed on heating, may be due to the presence of certain factors in *Jatropha* meal that mimic the action of lectin (agglutination of erythrocytes). The saponins, e.g., have been shown to produce such effects (Fenwick, *et al.* 1991).
Cyanogenic glucosides are the water-soluble components (James, et al, 1977). Glucosinolates are sulfur-rich, anionic natural products that are soluble in water and methanol (Fereidoon Shahidi, et al, 1988).

Phytic Acid is the 6 phosphates ester of inositol. It is found in almost grains and fibers of plants in the form of insoluble calcium and magnesium salts (called Phytin) which are considered as the storage of organic phosphorus in plants. The most outstanding feature of phytic acid is its powerful chelating function which can be adapted in biological, in foods as well as in industrial fields. It is soluble in water and slightly soluble in ethanol.
Flavonoids are aromatic compounds whose basic structure is a phenyl chroman skeleton (C6-C3-C6/A ring-C ring-B ring), and are classified into flavones, flavonols, flavanones, flavanonols, isoflavones, anthocyanins, flavanols, chalcones, aurones and the like in accordance with the difference in the C ring moieties. They are slightly soluble in water.

Amylase and trypsin inhibitors present in the seed are also soluble in water.

Vitexin is an **apigenin flavone glycoside**, Isovitexin (or homovitexin, saponaretin) is the apigenin-6-C-glucoside. Vitexin inhibits **thyroid peroxidase** thus contributing to **goiter**. They are soluble in ethanol.

### 5.1. Selection of different concentrations of the extracts:

By trial and error, different concentrations of all the solvent extracts of seed, oil and meal were tried on the four pests, *Helicoverpa armigera*, *Bemisia tabaci*, *Cnaphalocrosis medinalis* and *Diacrisia obliqua* and it was observed that all the pests were sensitive to 125 ppm, 250 ppm showing moderate results and 500 ppm being the most effective in 48 hours i.e., LC$_{100}$=500ppm in 48 hours.

The results demonstrate the insecticidal activity of all the tested preparations from the seeds of *Jatropha curcas* both in the field and lab levels which is discussed below.

### 5.2. Lab efficacies:
5.2.1. Lab efficacy of solvent extracts of Jatropha curcas seed on Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis and Diacrisia oblique.

The efficacy of all the solvent extracts of J. curcas seed was almost similar on all the test insects under laboratory conditions. Among the different concentrations tested, 500 ppm concentration resulted in significantly highest percent reduction in population (LC$_{100}$ value) on all the four pests i.e., Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis and Diacrisia obliqua after 48 hours of treatment. Corresponding untreated control showed no mortality. The percent reductions in population with methanol extracts of seed at 500ppm are 85.94% on third instar larva of Helicoverpa armigera, 65.95% on Adult whitefly, 80.03% on third instar larva of Rice leaf folder and 85.94% on third instar larva of Bihar hairy caterpillar.

Among different solvent extracts, tested methanol extract showed potent antifeedant and pesticidal activity which indicated that these extracts contained classes of compounds that can control different insects and they are the phorbol ester fraction of the oil present in the seed. Phorbol esters were enriched by extracting the seed three times with methanol. Similar observations were reported where the most important toxic compounds in extracts of J. curcas were found to be phorbol esters, which are enriched as hydrophobic molecules by methanol extraction (Hirota, et al. 1988). The phorbol esters are amphiphillic molecules hence they are soluble in all the
solvents up to certain extent which is responsible for the insecticidal activity of all the solvent extracts of the seed.

The toxic activity of phorbols may be due to interference with cell signal pathways. Phorbol esters mimic the activity of diacylglycerol in the activation of protein kinase C, which is part of the inositol phosphate/Ca\(^{2+}\) signal pathway. As diacylglycerol has two fatty acid side chains, at least one long fatty acid side-chain should be essential for pesticidal activity of phorbol esters. Pesticidal activity of phorbol esters is mediated through stimulation of protein kinase C and the ensuing degradation of the inositol phosphate/Ca\(^{2+}\) signal pathway. Aqueous extract of *J. curcas* seeds was much less toxic against the pests, possibly due to hydrophilic components in *J. curcas* such as saponins, curcin, phytates and protease inhibitors (Morgue, et al. 1961; Stripe, et al. 1976; Makkar, et al. 1997). The high levels of trypsin inhibitor, lectin and phytate content might aggravate adverse effects on the insect but do not contribute to the short term toxicity (Makkar, et al 1997). The toxic components of seed were also less soluble in the aromatic organic solvents like benzene and hence the results are poor.

5.2.2. Lab efficacy of solvent extracts of oil on *Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis and Diacrisia obliqua*
The efficacy of the all extracts of *Jatropha* oil was almost similar on all the species of test insects under laboratory conditions. The percent reductions in population with methanol extracts of oil at 500ppm are 85.94% on third instar larva of *Helicoverpa armigera*, 62.29% on Adult whitefly, 68.66% on third instar larva of Rice leaf folder and 71.56% on third instar larva of Bihar hairy caterpillar after 48 hours of treatment. Corresponding control treatments alone showed no insect mortality. Among all the solvent extracts, methanol and hexane extracts showed potent antifeedant and pesticidal activity which indicated that these extracts contained classes of compounds that can control different insects and they are exclusively the phorbol ester fraction of the oil. The oil of *J. curcas* seed contains the phorbol esters alone and it is the methanol extract again which is the potent preparation which is effective against all the pests. Phorbol esters are enriched as hydrophobic molecules by methanol extraction (Hirota, *et al*. 1988). The oil is best soluble in hexane and thus the phorbols too, which might be the reason for efficacy of hexane extract. The phorbol esters are amphiphillic molecules and they are soluble in all the solvents up to certain extent which is responsible for the insecticidal activity of all the solvent extracts of the seed.

The toxic components of seed were also less soluble in the aromatic organic solvents like benzene and hence the results are poor. All phorbol esters are extracted with the oil fraction. Thus the oil extracts of solvents are effective next to the seed extracts comparatively.
5.2.3. **Lab efficacy of solvent extracts of *Jatropha curcas* meal on *Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis* and *Diacrisia obliqua***

The fraction of seed obtained after the removal of oil is the meal which still contains traces of oil. The phorbol ester content depends on the residual oil present in the meal after processing. The efficacy of the all the solvent extracts of *J. curcas* seed meal was almost similar on all the tested pests under laboratory conditions. The percent reductions in population with methanol extracts of meal at 500ppm are 85.94% on third instar larva of *Helicoverpa armigera*, 63.54% on Adult whitefly, 78.2% on third instar larva of Rice leaf folder and 85.9% on third instar larva of Bihar hairy caterpillar. The phorbol esters are amphiphilic molecules but the extracts of methanol were the most effective followed by hexane, ethanol, petroleum ether, ethyl acetate extracts and least effect was observed with aqueous and benzene extracts at 500ppm after 48 hours of treatment. Corresponding untreated control treatments showed no insect mortality. Phorbol esters are enriched as hydrophobic molecules by methanol extraction (Hirota, *et al.* 1988). They are also soluble in most organic solvents. The meal also contains the saponins, lectin (curcin, toxalbumin), phytates, amylase and protease inhibitors (Morgue, *et al.* 1961; Stripe, *et al.* 1976; Makkar, *et al.* 1997). Saponins are amphipathic and the other components are mostly hydrophilic and are present in the aqueous and ethanol extracts to some extent which might be the reason for their effect on the pests. But the high levels of trypsin inhibitors, lectin and phytate content might aggergate adverse effects.
but do not contribute to the short term toxicity (Makkar, *et al* 1997). The phorbol acts as analogue for diacylglycerol and is a stronger protein kinase C activator that is hardly metabolized by cell (Segal, *et al*., 1975).

Since most of the phorbol esters are extracted with the oil fraction. Thus comparatively, the meal extracts of solvents are effective only next to the seed and oil extracts.

5.3. **Field efficacies:**

5.3.1. **Field efficacy of solvent extracts of *Jatropha curcas* seed on *Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis* and *Diacrisia oblique*.  

Among the different solvent extracts of *J. curcas* tested under field conditions methanol extract resulted in significantly highest percent reduction mortality over control compared to rest of the treatments. Among the different concentrations tested 500 ppm concentration resulted in significantly highest percent reduction in population (LC$_{100}$ value) on all the four pests on their respective crops i.e., *Helicoverpa* on musk melon, *Bemisia tabaci* on tomato, *Cnaphalocrosis medinalis* on rice and *Diacrisia obliqua* on ground nut, compared to the 250ppm showing moderate mortalities and 125ppm showing poor results. The extracts of hexane and methanol were the most effective followed by ethanol, petroleum ether, ethyl acetate extracts and less effect was observed with aqueous and benzene extracts after 72 hours in the field. The percent reductions in population with methanol extracts of seed at 500ppm are 82.8% on third instar larva of
Helicoverpa armigera, 64.7% on Adult whitefly, 76.4% on third instar larva of Rice leaf folder and 83.1% on third instar larva of Bihar hairy caterpillar.

The oil of J. curcas seed contains the phorbol esters which are enriched as hydrophobic molecules by methanol extraction (Hirota et al. 1988). As the phorbol esters are amphiphilic molecules, they are soluble in all the solvents and thus all the extracts showed mortalities of all the insects to some extent. The meal also contains the saponins, lectin (curcin, toxalbumin), phytates, amylase and protease inhibitors (Morgue, et al. 1961; Stripe, et al. 1976; Makkar, et al. 1997). Saponins are amphipathic and the other components are mostly hydrophilic which might be the reason for their effect of all extracts on all the pests.

When the different concentrations of the solvent extracts were sprayed on the crop, the pests on the respective crop were also in direct contact with the sprays. The most important toxic compounds in extracts of J. curcas are likely to be phorbol esters, which are enriched as hydrophobic molecules by methanol extraction (Hirota et al. 1988). Another observation is the formation of vesicles on the surfaces of the pests. This observation is supported by the investigations of Rug and Ruppel (2000).
Fig. 5.3: Inflammatory responses induced by phorbol esters

(Source: www.bioweb.wku.edu).

Phorbol esters are known to directly activate protein kinase C (PKC) (Castagna, et al. 1982). This key enzyme of signalling cascades plays a critical role in maintaining the integrity of the insect surface (Wiest, et al. 1994). Activation of PKC by phorbol esters may lead to phosphorylation of different proteins and a consequent reorganization of the cell cytoskeleton (Bershadsky, et al. 1990). Protein kinase C also regulates the activity of ion channels, which may lead to vesicle formation on the insect surface, as observed for schistosomes treated with praziquantel (Xiao, et al. 1984) or with a pore-forming toxin (Ruppel, et al. 1987). Thus it can be hypothesized that the phorbol esters in extracts of J. curcas induce osmolaric instability, surface vesiculation and subsequent death of the pests.

5.3.2. Field efficacy of solvent extracts of Jatropha curcas oil on Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis and Diacrisia obliqua
The efficacy of the all extracts of oil was almost similar on all the tested pests under laboratory and field conditions. The extracts of hexane and methanol were the most effective followed by ethanol, petroleum ether, ethyl acetate extracts and least effect was observed with aqueous and benzene extracts at 500ppm in 72 hours for the complete mortality of the pests taken (n=20) i.e. LC\textsubscript{100} values. Corresponding untreated control showed no insect mortality. The percent reductions in population with methanol extracts of oil at 500ppm are 70.26% on third instar larva of *Helicoverpa armigera*, 60.5% on Adult whitefly, 70.38% on third instar larva of Rice leaf folder and 64.08% on third instar larva of Bihar hairy caterpillar. Among all the solvent extracts, methanol and hexane extracts showed potent antifeedant and pesticidal activity which indicated that these extracts contained classes of compounds that can control different insects and they are exclusively the phorbol ester fraction of the oil. The oil of *J. curcas* seed contains the phorbol esters alone and it is the methanol extract again which is the potent preparation which is effective against all the pests. Phorbol esters are enriched as hydrophobic molecules by methanol extraction (Hirota, *et al*. 1988).

The toxic components of seed were also less soluble in the aromatic organic solvents like benzene and hence the results are poor. All phorbol esters are extracted with the oil fraction. Thus the oil extracts of solvents are effective next to the seed extracts comparatively.

Phorbol esters are known to directly activate protein kinase C (PKC) (Castagna, *et al*. 1982). This key enzyme of signalling cascades plays a
critical role in maintaining the integrity of the insect surface (Wiest, et al. 1994). Activation of PKC by phorbol esters may lead to phosphorylation of different proteins and a consequent reorganization of the cell cytoskeleton (Bershadsky, et al. 1990). PKC also regulates the activity of ion channels thereby leading to vesicle formation on the insect surface (Xiao, et al. 1984) or with a pore-forming toxin (Ruppel, et al. 1987). Thus, it can be concluded that the phorbol esters in extracts of J. curcas induce osmolaric instability, surface vesiculation and subsequent death of the pests.

5.3.3. **Field efficacy of solvent extracts of Jatropha curcas meal on Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis and Diacrisia obliqua**

The fraction of seed obtained after the removal of oil is the meal which still contains traces of oil. The phorbol ester content depends on the residual oil present in the meal after processing. The efficacy of the all the solvent extracts of J. curcas seed meal was almost similar on all the tested pests under laboratory conditions. The phorbol esters are amphiphillic molecules, soluble in all solvents. But the extracts of methanol were the most effective followed by ethanol, hexane, petroleum ether, ethyl acetate extracts and least effect was observed with aqueous and benzene extracts at 500ppm in 72 hours after the treatment for the complete mortality of the test insects i.e. LC$_{100}$ values. The percent reductions in population with methanol extracts of meal at 500ppm are 74.9% on third instar larva of Helicoverpa armigera, 60.5% on Adult whitefly, 70.82% on third instar larva of Rice leaf folder and 76.9% on third instar larva of Bihar hairy caterpillar.
Corresponding untreated control treatments showed no mortality. Phorbol esters are enriched as hydrophobic molecules by methanol extraction (Hirota, et al. 1988). They are also soluble in most organic solvents. The meal also contains the saponins, lectin (curcin, toxalbumin), phytates, amylase and protease inhibitors (Morgue, et al. 1961; Stripe, et al. 1976; Makkar, et al. 1997) which enter into the solvents and show the insecticidal effect.

The toxic activity of phorbols may be due to interference with cell signal pathways. Phorbol esters not only mimic the activity of diacylglycerol in the activation of protein kinase C further leading to a cascade of events, they also cause surface vesiculation and subsequent death of the pests. Comparatively, the meal extracts of solvents are effective only next to the seed and oil extracts.

Jatropha seed oil and isolated phorbol esters were employed in a human cell culture system and could not detect evidence for mutagenic or genotoxic effects (Wink, 1993).

5.4. **Effect of solvent extracts of Jatropha curcas seed, oil and meal on enzymes (Amylase, Protease and Lactate Dehydrogenase) activities in Helicoverpa armigera.**

5.4.1. **Effect of solvent extracts of Jatropha curcas seed on amylase activities in Helicoverpa armigera**

Amylase is an enzyme of carbohydrate metabolism which cleaves the glycosidic linkages in amylose. Among the different solvent types tested,
methanol extract at all the three concentrations was found to be most effective in reducing the amylase activity (0.93, 0.97 and $0.57 \times 10^4 \mu g/mg/min$ respectively at 125, 250 and 500 ppm) of larva. Among the different solvent types tested, methanol at 500 ppm was found to be the most effective combination on reducing the amylase activity ($0.57 \times 10^4 \mu g/mg/min$) in larva studied under lab conditions. The oil of *J. curcas* seed contains the phorbol esters. The most important toxic compounds in extracts of *J. curcas* are likely to be phorbol esters, which are enriched as hydrophobic molecules by methanol extraction (Hirota, *et al.* 1988). The effect of mechanism of phorbol esters on PKC whose action is altered which decreases the activity of amylase is explained later in the same chapter.
5.4.2. Effect of solvent extracts of *Jatropha curcas* oil on amylase activities in *Helicoverpa armigera*

Among the different solvent types tested, methanol extract was found to be most effective in reducing the amylase activity at all the three concentrations tested (1.08, 0.74, and $0.67 \times 10^4 \, \mu g/mg/min$) of larva and methanol at 500 ppm was found to be the most effective combination on reducing the amylase activity ($0.67 \times 10^4 \, \mu g/mg/min$) in larva studied under lab conditions. The oil of *J. curcas* seed contains the phorbol esters alone and it is the methanol extract again which is the potent preparation which is effective against all the pests. The main reason is the enrichment of phorbols with methanol and their effect on PKC whose action is altered and this mechanism is explained later in the same chapter.

5.4.3. Effect of solvent extracts of *Jatropha curcas* meal on amylase activities in *Helicoverpa armigera*

The fraction of seed obtained after the removal of oil is the meal which still contains traces of oil. The phorbol ester content depends on the residual oil present in the meal after processing.

Among the different solvent types tested, extract of methanol with meal was found to be more effective in decreasing the activity of amylase. Among the different solvent types tested, extract of methanol was found to be more effective in decreasing activity of amylase ($2.16 \times 10^4 \, \mu g/mg/min$). Among the different concentrations tested for different extracts 500 ppm was most effective in reducing the amylase activity, whereas 125 and 250
ppm are on par and amylase activity did not significantly differ with different solvent extracts at 125 ppm. The reason is the enrichment of phorbols with methanol and their effect on PKC whose action is altered and curcin in the aqueous extracts and these mechanisms are also explained later in the same chapter.

**Effect of solvent extracts of *Jatropha curcas* seed on protease activities in *Helicoverpa armigera***

Protease is an enzyme which cleaves the peptide bonds in the polypeptide chains in the proteins. Among the different solvent types tested, extract of methanol with seed was found to be more effective in decreasing the activity of protease at all the concentrations tested (2.11, 2.06 and 1.25 ×10^4 µg/mg/min) compared to the remaining extracts and control. By increasing the concentrations from 125 ppm to 500 ppm protease activity was reduced significantly for all the extracts. It is due to the phorbols which are enriched with methanol and their activity as analog of DAG to the receptor, PKC.

**Effect of solvent extracts of *Jatropha curcas* oil on protease activities in *Helicoverpa armigera***

Among the different solvent types tested at different concentration, extract using methanol with oil of *J. curcas* was found to be more effective in decreasing the protease activity (2.45, 2.39 and 1.45 × 10^4 µg/mg/min at 125, 250 and 500 ppm respectively) of larva compared to control. Among the three different concentrations tested 500 ppm was found to be most effective (0.57 × 10^4 µg/mg/min) in larva studied under lab conditions. It is
because of the phorbol ester portion of oil which results in decrease of activity of protein digesting enzyme, protease.

**Effect of solvent extracts of *Jatropha curcas* meal on protease activities in *Helicoverpa armigera***

Among the different solvent types tested, extract of methanol and ethanol from meal of *J. curcas* was found to be more effective in decreasing the activity of protease (5.96 and 5.98 × 10⁴ µg/mg/min respectively) of larva compared to control. Among the three different concentrations tested 500 ppm was more effective in reducing protease activity compared to 125 and 250 ppm that showed no significant decrease in enzyme activity. The effect of phorbol esters depends on the percentage of oil that is left over in the meal portion of the seed.

**5.6.1. Effect of solvent extracts of *Jatropha curcas* seed on Lactate dehydrogenase activities in *Helicoverpa armigera***

Among the different solvent types tested, methanol extraction was found to be most effective in reducing LDH activity at all the three concentrations (6.44, 6.37 and 2.80 × 10⁴ µg/mg/min compared to rest of the extracts. Phorbol esters in the oil portion of seed are enriched with methanol (Hirota, *et al.* 1988). It is the indirect effect shown by the porbol esters which leads to the decrease in the specific activities of enzymes.

**5.6.2. Effect of solvent extracts of *Jatropha curcas* oil on lactatedehydrogenase activities in *Helicoverpa armigera***
LDH is an enzyme in carbohydrate metabolism involved in the conversion of pyruvate to lactate. Among the different solvent types tested at three different concentrations, methanol extraction was found to be most effective in reducing LDH activity ($7.47, 7.58$ and $4.42 \times 10^4 \mu g/mg/min$ at 125, 250 and 500 ppm respectively) over control. Methanol extract at 500 ppm was more effective to reduce LDH activity ($4.42 \times 10^4 \mu g/mg/min$).

Phorbol esters are enriched with methanol (Hirota, et al. 1988) which show the indirect effect which leads to the decrease in the specific activities of enzymes.

5.6.3. Effect of solvent extracts of *Jatropha curcas* meal on lactate dehydrogenase activities in *Helicoverpa armigera*

Among the different solvent types tested, methanol extraction used at highest concentration was found to be most effective in reducing LDH activity ($8.20 \times 10^4 \mu g/mg/min$) compared to control. Phorbol esters are enriched with methanol (Hirota, et al. 1988) which show the indirect effect which leads to the decrease in the specific activities of enzymes. The effect depends on the left over oil proportion in the meal.

Under laboratory conditions different solvent extracts of *J.curcas* seed, oil and meal tested, methanol extract resulted in significantly highest reduction in specific activities of amylase, protease and lactate dehydrogenase over control followed by the extracts of ethanol, petroleum ether, aqueous extract, ethyl acetate and benzene. The order of affectivity is seed followed by oil and meal extracts. Among the different concentrations tested, 500 ppm concentration resulted in significantly the highest reduction
in specific activity of amylase, protease and lactate dehydrogenase in *Helicoverpa armigera*, third instar larvae (*n*=10). Meal and oil extracts of *J. curcas* at 125 ppm showed no effects on the activities of amylase protease and lactate dehydrogenase which indicates that the concentrations of phorbol esters at 125 ppm are not sufficient for the effects to be seen.

Phorbol esters are enriched with methanol (Hirota, *et al*. 1988). It may be the indirect effect shown by the phorbol esters that lead to a decrease in the specific activities of enzymes.

One of the major observations in these investigations is that all the extracts are potent antifeedants on the larvae. Similar observations were reported by Adebowale and Adedire, 2006. Antifeedants are the substances which, when tasted by insects, results in either temporarily or permanently cessation of feeding, depending on potency, (Klocke, *et al*. 1989). There are different modes of actions existing for antifeedants. One of the modes of action by which secondary plant compounds act is ‘toxicity’. Once the toxicants are digested by the insect they work by disrupting cellular, biochemical, and physiological processes (Mendel, *et al*.1991).

*Jatropha* seed extracts are one such antifeedants with toxic compounds such as phorbol esters and curcin. One of the causes for antifeedant behavior is the decrease in activities of digestive enzymes (Klocke, *et al* 1989) and thus three enzymes are taken for the investigation i.e. amylase, protease and a general enzyme present in every cell of the body, lactate dehydrogenase that is involved in carbohydrate metabolism. The mechanism of phorbol esters in reducing the specific activities of
enzymes is as follows. The primary action of phorbol esters is on biological membranes.

The phorbol esters are amphiphilic molecules and have tendency to bind to phospholipid membrane receptors. These receptors are usually the primary targets for the phorbol esters. The initial membrane effects include modification in activities of cell receptors. Phorbol esters generally bind to the receptor of protein kinase C. The PKC family belongs to a group of kinases that phosphorylates serine or threonine residue of the substrate protein by transfer of the -phosphate group of ATP onto a hydroxyl group of serine or threonine.

Activation of protein kinase C (PKC) by diacylglycerol generated through hydrolysis of phosphatidylinositol 4, 5-bisphosphate is a classic signal transduction mechanism. The most investigated activity of the phorbol is its binding and activation of PKC, which plays a critical role in signal transduction pathway. (Clemens, et al.1992; Nishizuka, 1992). It has been proposed that the phorbol esters convert PKC into a constitutive active form that is irreversibly inserted into the membrane (Mosior and Newton, 1995). During normal signal transduction, the enzyme is activated by DAG (diacylglycerol), which is then rapidly hydrolyzed. DAG is responsible for activating PKC function by increasing its affinity for phosphatidylserine (PS)-containing membranes. Upon activation, PKC enzymes are translocated to the plasma membrane by RACK (receptor for activated C-kinase) proteins (membrane-bound receptor protein for activated PKC) to conduct various other signal transduction pathways.
The phorbol acts as analogue for DAG and is a stronger PKC activator that is hardly metabolized by cell (Segal, et al. 1975). It hyperactivates PKC and triggers cell proliferation, thus amplifying the efficacy of carcinogens. These phorbols can both activate PKC and after longer incubation down-regulate the enzyme (Silinsky and Searl 2003). The regulation of PKC by DAG and phorbols occur by the same mechanism, with some differences in the strength of interaction (Mosior and Newton 1995). DAG, with the help of divalent cations like calcium, activates protein kinase C which in turn phosphorylates many proteins. Instead of DAG, when phorbol esters bind to PKC, the entire mechanism is altered and the proteins are not phosphorylated i.e the enzymes which are chemically proteins are not phosphorylated as a result of which they are not activated and thus, their activities are lowered. It may also alter the feeding behaviour of the insect toward food avoidance and maintain body metabolism at the expenses of storage or cellular proteins, however, avoided dietary practice may be resumed adaptively. Once the enzyme activities are lowered, metabolic disturbances take place in the pest, thereby leading to the death of the pest. It has been observed a sharp decrease in the activity of digestive enzymes as a result of food deprivation and this response depends on the enzyme (Cox and Willason, 1981, Mayzaud and Mayzaud, 1985).

Several studies have shown that feeding is necessary for the stimulation of enzyme activities (Sibley, 1981; Broadway and Duffey, 1988).
It is evident that exposure to botanical insecticides in larval diet has significant effects on several enzyme activities found in the third instar larvae of *Helicoverpa*. (Smirle, et al., 1996).

Changes in metabolism, physiology and decrease in gut enzyme activities of the treated pests may be expected to affect enzyme activities (Schmutterer, 1990, Mordue and Blackwell, 1993). The doses of *Jatropha* seed extracts affected the amylase, protease and LDH activities of the *Helicoverpa armigera* larvae. The results in the present investigation support this hypothesis. LDH is involved in the production of energy being particularly important when a considerable amount of additional energy is rapidly required. A negative correlation between LDH activity and oxygen levels has been reported for some aquatic organisms suggesting a possible adjustment in response to lowered oxygen levels (Augenfeld, 1966; Bidlack and Lockshin, 1976, Diamantino, et al., 2001). This probably occurs also in chemical stress. Therefore, this enzyme may be a sensitive criterion for pesticide exposure (Diamantino, et al., 2001., Wu and Lam, 1997). Decrease in LDH activity results in accumulation of pyruvate and that indicates reduced metabolism.

Digestive proteases play critical role in an insect's physiology—breaking down proteins into amino acids essential for growth and development (Terra, et al., 1996). Disruption of these processes has the potential to suppress populations of phytophagous insect pests by limiting nutrient availability. Reduced amylase activities result in the accumulation of amylase and thereby resulting in carbohydrate metabolic imbalance.
The aqueous extracts of *Jatropha* seed and meal contain curcin, a lectin and a toxalbumin which is also another reason for decrease in enzyme activities and death of the *Helicoverpa armigera*. Though curcin shows very less short term toxicity, its effect can be explained as below.

Curcin is a Ribosome-inactivating protein (RIP), a toxin that can inhibit protein synthesis in eukaryotic cells by catalytically damaging ribosomes (Barbieri, *et al*, 1993) with rRNA N-glycosidase activity. It inhibits protein synthesis by interfering with the binding of the 28S RNA to elongation factors (Peumnas, *et al* 2001). Also the synthesis of enzymes (chemically proteins) is inhibited. The results in the present investigation are supportive to the statement that aqueous extracts of *Jatropha curcas* seed shows insecticidal effects against crop pests such as *Helicoverpa armigera* and *Sitophilus zeamays* (Solsoley, 1995). Curcin contains one cysteine residue; it may directly form a disulphide bond with an activated antibody (Lin, *et al* 2010). A disulphide linkage is usually thought to be essential for maximal cytotoxicity. Most of types I RIPs have not any free cysteine residue. Therefore, the curcin is a novel protein, and RIP of containing cysteine, which might be ideal for the preparation of immunoconjugates with great potential as a chemotherapeutic agent for the treatment of various cancers or AIDS.

Based on this extensive study both in the field and laboratory conditions, including various enzyme activities the following conclusions were drawn.
Jatropha curcas seed can be used as a potential botanical pesticide for the control of Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis and Diacrisia obliqua.

- Jatropha curcas seed can be used as a potential botanical pesticide for the control of Helicoverpa armigera, Bemisia tabaci, Cnaphalocrosis medinalis and Diacrisia obliqua.
- Extraction method and solvent used to prepare solution for spraying may be applied depending upon the cost effectiveness and availability.
- Solvents such as methanol and ethanol may be used effectively for industrial manufacturing of botanical insecticide.
- Even the aqueous extracts (using water) may be used where instead of meal, oil or seed may be suggested for farmer level preparations.