Post-harvesting losses is a critical component for filling the demands of ever increasing population. Because a large number of food security issues can be facing in the future. Current study was planned to probe the comparative insecticidal, growth inhibitory and feeding detterent activities of spinetoram, chlorfenapyr, cypermethrin and beta-cyfluthrin against *Trogoderma granarium* (Everts) and *Tribolium castaneum* (Herbst) under laboratory conditions. The insecticides were used at three different concentrations i.e., 5, 7 and 9ppm. Results revealed that maximum adult mean percent mortality of *T. castaneum* was recorded at highest concentration (9ppm) was 78.08% followed by 69.41% at 7ppm and 61.41% at 5ppm. In case of *T. granarium* at highest concentration (9ppm) the mortality was 72.58% followed by 64.08% at 7ppm and 55.33% at 5ppm. Results regarding growth inhibition showed that cypermethrin and chlorfenapyr gave highest values 28.77 and 23.78% for larval emergence inhibition. While beta-cyfluthrin gave lowest larval emergence inhibition against the *T. castaneum*, beta cyfluthrin gave 53.02% pupae inhibition. 50.26%, 48.66% and 46.48% pupae inhibition values were given by spinetoram, chlorfenapyr and cypermethrin, respectively. Adult emergence inhibition was highest 40.17% in case of cypermethrin followed by chlorfenapyr (30.60%). Similarly, the efficacy of all tested insecticides in term of feeding deterrence for both insects was cypermethrin > chlorfenapyr > spinetoram > beta-cyfluthrin.

## Toxicity of four Cuban botanical derivatives against two stored-products coleopteran pests

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### Abstract

Plants are a source of substances for protection of stored products. The Cuban flora has not yet been fully studied as a source of pesticides for postharvest protection, partly due to its great diversity. The toxicity of four Cuban plant derivatives against *Lasioderma serricorne* (F.) and *Sitophilus zeamais* Motschulsky was investigated. The anti-insect activity of the powders and the essential oil from plants belonging to Asteraceae, Fabaceae and Piperaceae was tested. Mortality and emergence of adult insects and the repellent effect of products were evaluated. Two products derived from *Piper aduncum* subsp. *ossanum*, caused high mortality (81,6 and 100%), reduced emergence (27,9 and 0,4%) and exhibited strong repellent activity on *L. serricorne*. Against *S. zeamais*, treatments with the highest mortality values were stems of *Lonchocarpus punctatus* (72,4%), seeds and stems of *Canavalia ensiformis* (64,9 and 69,9%), and leaves of *Tithonia diversifolia* (67,2%). The progeny production of *S. zeamais* was inhibited by powders of *L. punctatus* stems (31,8%), *C. ensiformis* seeds (40,5%), leaves (43,7%) and stems (30,6%), and *T. diversifolia* leaves (38,7%). The stems of *C. ensiformis*, leaves of *T. diversifolia* and *L. punctatus* had the highest repellent effect. These products have potential for small-scale treatments of grains for protection against both insects, and *P. aduncum* subsp. *ossanum*-based products to control *L. serricorne* infestation in tobacco. Identification of local candidates to develop effective and safe pesticides offers new alternatives to the Cuban agriculture in the control of storage pests.

**Keywords:** *Lasioderma serricorne*, *Sitophilus zeamais*, Fabaceae, Asteraceae, Piperaceae.

### Introduction

Stored products of agricultural and animal origin are attacked by many species of insect pests causing quantitative and qualitative losses and insect contamination in food commodities is an important quality control problem of concern for food industries (Rajendran and Sriranjini, 2008). Storage insects cause significant losses for grain and legume producers, due to the reduction of the quantity and quality of food for domestic consumption and the value of the grain for sale in the market (Jones et al., 2018). Concerns over the harmful effects of synthetic insecticides have stimulated interest in alternative pest management tactics including the use of botanical insecticides that provide novel modes of action against pests that have developed resistance against synthetic insecticides (Amoabeng et al., 2018).

Plants are a source of substances for protection of stored products (Ogendo et al., 2012). Plant parts and botanical derived products have gained importance because flora biodiversity has provided an excellent source of biologically active constituents or allelochemicals for use in traditional crop protection. In the tropics, plant biodiversity has provided an excellent source of allelochemicals for crop protection in traditional agriculture for centuries and crude local products were applied to crops and stored food grains to protect them from an array of pest species (Gahukar, 2014).

Cuba is considered as one of the most biodiverse countries in the world in terms of sheer numbers of species and has the richest plant biodiversity of all the islands in America, with an estimated 6,600 plant species of which 50% are endemic (González-Torres et al., 2013). The Cuban flora has not yet been fully studied as a source of pesticides for postharvest protection, partly due to its great diversity. To date, only a small fraction of the plant species has undergone systematic phytochemical or biochemical research, leaving valuable sources for commercial products undiscovered (Pino et al., 2013). The toxicity of four Cuban plant derivatives, *Tithonia diversifolia* (Hemsl.), *Lonchocarpus punctatus* Kunth, *Canavalia ensiformis* L. and *Piper aduncum* subsp. *ossanum* (C.DC.) Saralegui, against *Lasioderma serricorne* (F.) and *Sitophilus zeamais* Motschulsky was investigated.

## Materials and Methods

Plant derived products (powders and essential oil)

Raw plant materials were collected from four botanical species belonging to Asteraceae, Fabaceae and Piperaceae in Mayabeque, Cuba (Table 1).

**Tab. 1** Plant Material.

| Plant Family | Plant species | | Part of the plant |
|---|---|---|---|
| | Scientific name | Common name | |
| Asteraceae | *Tithonia diversifolia* (Hemsl.) | tree marigold, Mexican tournesol, Mexican sunflower, Japanese sunflower or Nitobe chrysanthemum | Leaf, stem, root |
| Fabaceae | *Lonchocarpus punctatus* Kunth | dotted lancepod | Leaf, stem, fruit |
| Fabaceae | *Canavalia ensiformis* L. | jack-bean | Leaf, stem, seed |
| Piperaceae | *Piper aduncum* subsp. *ossanum* (C.DC.) Saralegui | spiked pepper, canilla de muerto, guayuyo or platanillo de Cuba | Leaf |

Asteraceae and Fabaceae plant materials were dried in an oven at 45 ºC, then grounded into powder (< 1 mm) and sieved (250 µm). They were kept at room temperature in nylon bags and were identified according to the plant structures until use for bioassays. The essential oil of *P. aduncum* subsp. *ossanum* was extracted by hydrodistillation using a Clevenger type apparatus. After extraction, the essential oil was dried over anhydrous sodium sulphate and stored in a refrigerator at 4 ºC. Two powder formulations, PAO-1 and PAO-2, were obtained from the fresh leaf powder and the essential oil of *P. aduncum* subsp. *ossanum*, respectively. The anti-insect effect of products from *P. aduncum* subsp. *ossanum* (PAO-1, PAO-2) and zeolite, as an inert material, was tested against *L. serricorne* on chickpea. The biological activity of the nine powders from the different parts of *L. punctatus*, *C. ensiformis* and *T. diversifolia* (Table 1) was evaluated against *S. zeamais* on maize. Mortality and emergence of adult insects and the repellent effect of products were evaluated.

Insects

*Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) was reared on maize (*Zea mays* cv. Manitou) in controlled conditions (25 ± 2 ºC, 70 ± 5 % of relative humidity and 12:12 light/dark photoperiod) in the Institute of Animal Science, Mayabeque, Cuba. *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) was maintained on chickpea (*Cicer arietinum* L.), at 25,7 ± 1,95°C and 76,4 ± 9,18% r.h. under a 16:8 light/dark photoperiod, at the Entomology Laboratory, Agricultural Pests Group, National Centre of Animal and Plant Health, Mayabeque, Cuba. Experiments were conducted using emerged adults (age < 10 days old) from laboratory colonies.

Effect on adult mortality and F1 adult *emergence*

The effect of the derived plant products on adult mortality and F1 adult emergence was evaluated by grain treatment test (Silva et al., 2003). The grains of chickpea or maize (100 g) were mixed thoroughly with the products (plant powder (1g), PAO-1 (1g), PAO-2 (2g) or zeolite (2g)) in 1L glass jars. Twenty couples of adult beetles (*L. serricorne* or *S. zeamais*) were introduced into each glass jars containing the different treatments. Untreated grains in glass jars, where twenty insects were also placed, acted as the controls.

Adult mortality was evaluated 15 days after jar infestation for both insects. They were considered dead when no movement was observed after carefully touching them with a dissection needle. The live and dead *L. serricorne* adults were also counted after 3, 6, 9, and 12 days.
The number of emerged F1 adults (F1 progeny) were counted 58 days and 55 days after infestation with *L. serricorne* and *S. zeamais,* respectively. The percentage of insect emergence was calculated..

Repellent effect of plant products to the two betle species

The effect of the tested materials applied to the grains on the behaviour of the insects was established by using an arena formed by five circular plastic boxes (8,5 cm of diameter, 1.5 cm of height). The central box was connected with the rest using plastic tubes disposed diagonally. The boxes with treated grains (the same concentration used in previos bioassay) and the control (untreated grain), were distributed in two symmetrically opposed boxes. In the central container, 50 adults of *L serricorne* or S. zeamais were released. After 24 hours, the number of insects was counted in each box and the index of repellence (IR) was calculated (Mazzonetto and Vendramim, 2003):
$IR = 2G/(G + P)$
Where,
G =percentage of insects in the treatment
P = percentage of insects in the control
The effect of the product was clasified: neutral if IR=1, attractive if IR>1 and repellent if IR<1.

Experimental design and statistical analysis

For both bioassays, all treatments were arranged in a completely randomized design in the laboratory and kept at the same conditions asthe colonies. Each treatment was replicated four (adult mortality and emergence bioassay) or three (repellency bioassay) times.

The mortality counts were corrected with Abbott's formula (Abbott, 1925). All data were subjected to analysis of variance (ANOVA) and the means of treatments were compared by Duncan's Multiple Range Test at p < 0,05. The Statistical Analysis System (SAS Version 9.2) was used.

**Results**

The two products derived from *P. aduncum* subsp. *ossanum* caused high mortality and reduced F1 emergence of *L. serricorne* (Table 2). The highest values of mortality and reduction of adult emergence were observed with PAO-2; although, in the variable mortality, no significant differences were found with the other treatments.

**Tab. 2** Effect of products from *Piper aduncum* subsp. *ossanum* on mortality and F1 emergence of the *Lasioderma serricorne* adults.

| Treatment | Mortality (%)* | No. of emerged insects (Mean ± EE)* | Emergence (%) |
|---|---|---|---|
| PAO-1 | 81,58[a] | 87,25 ± 37,7[b] | 27,88 |
| PAO-2 | 100[a] | 1,25 ± 0,75[a] | 0,40 |
| Zeolite | 94,74[a] | 50 ± 21,46[b] | 15,97 |
| Control | | 313 ± 25,89[c] | 100 |

*15 days, four replicates of 20 insects in each replication, means in the same column with letters in common do not differ significantly (Duncan's Multiple Range Test, $p > 0.05$)

Contact toxicity assayed by coating chickpea grains showed that both products caused mortality of *L. serricorne* and it was time of exposure dependent (Figure 1). PAO-2 had the fastest action, causing the highest number of deaths (43.33%) after three days. Treatment with PAO-1 induced death to increase from the ninth day (46%). Mortality due to the zeolite increased from the sixth day, with values higher than those obtained with the other treatments after nine days.

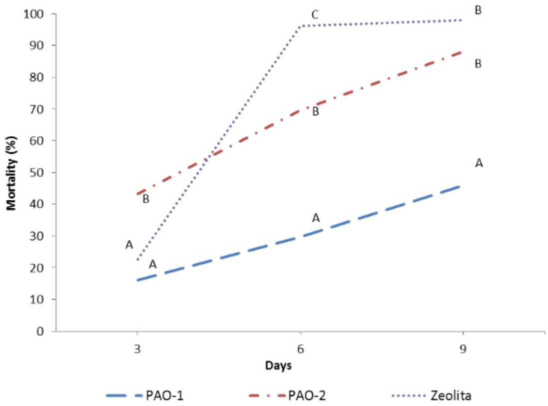


**Fig. 1** Mortality of *Lasioderma serricorne* produced by products of *Piper aduncum* subsp. *ossanum* and zeolite after different periods of treatment.

All the products tested on *L. serricorne* showed a repellent effect, with IR values less than one (Table. 3). The best result was obtained applying PAO-1 with a value of 0.27.

Out of the nine treatments tested against *S. zeamais*, six surpassed 40% of mortality and five presented values below 50% of emergence from F1 in comparison to the control (Table 4).

**Tab. 3** Effect of products from *Piper aduncum* subsp. *ossanum* on behavior of the *Lasioderma serricorne* adults.

| Treatments | Index of repellence (IR) | Classification |
|---|---|---|
| PAO-1 | 0,27 | Repellent |
| PAO-2 | 0,80 | Repellent |
| Zeolite | 0,68 | Repellent |

The treatments with the highest mortality values were stems of *Lonchocarpus punctatus*, seeds and stems of *Canavalia ensiformis*, and leaves of *Tithonia diversifolia*. Regarding the percent of F1 reduction, the best treatments were powders of: *L. punctatus* stems, *C. ensiformis* seeds, leaves and stems, and *T. diversifolia* leaves.

**Tab. 4** Effect of powders from *Tithonia diversifolia, Lonchocarpus punctatus* and *Canavalia ensiformis* on mortality, F1 emergence and behavior of the *Sitophilus zeamais* adults.

| Treatment | Mortality (%)* | Emergence (%) | Index of repellence (IR)** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| *T. diversifolia* (leaf) | 67,20[a] | 38,7[fe] | 0,47 |
| *T. diversifolia* (stem) | 45,87[b] | 50,8[de] | 0,72 |
| *T. diversifolia* (root) | 22,10[cd] | 78,6[b] | 1,12 |
| *L. punctatus* (leaf) | 25,16[cd] | 60,12[cde] | 0,48 |
| *L. punctatus* (stem) | 72,36[a] | 31,79[f] | 0,71 |
| *L. punctatus* (fruit) | 19,49[ed] | 82,12[b] | 0,82 |
| *C. ensiformis* (leaf) | 46,81[b] | 43,75[fde] | 0,84 |
| *C. ensiformis* (stem) | 69,93[a] | 30,56[f] | 0,67 |
| *C. ensiformis* (seed) | 64,93[a] | 40,48[fde] | 0,93 |

15 days, four replicates of 20 insects in each replication, means in the same column with letters incommon do not differ significantly (Duncan's Multiple Range Test, $p > 0.05$)
[**]Classification neutral if IR=1, attractive if IR>1 and repellent if IR<1

The index of repellence attained values below the unit for all of tested materials against *S. zeamais*, except for roots from *T. diversifolia* (Table 4). The leaves of *T. diversifolia* and *L. punctatus* and the stems of *C. ensiformis* had the highest repellent effect.

## Discussion

Botanical derivatives, with biological activity against insects, include compounds with behavioral actions – those causing repellence, feeding deterrence or oviposition deterrence – and those with physiological actions – those causing acute toxicity, developmental disruption or growth inhibition. It is not uncommon for a plant secondary compound to have both behavioral and physiological effects in one insect species, or different effects in different species (Isman, 2014).

The products of *P. aduncum* subsp *ossanum* demonstrated the anti-insect properties of the plants of the Piperaceae family. According to Silva et al., (2003), 40% mortality must be achieved to consider a treatment promising. The activity of the *P. aduncum* essential oil was studied against *Tenebrio molitor* L and *Callosobruchus maculatus* (F.), it showed insecticidal activity against both insects (Fazolin et al., 2007, Pereira et al., 2008). The *P. aduncum* subsp. *ossanum* is an endemic subspecie, whose production of secondary metabolites is different from that of the plants of this species growing in other geographical regions, even in the same country (Pino et al., 2011). To the best of our knowledge, the toxicity of its products against *L. serricorne* has not been previously reported.

From a chemical point of view, the *P. aduncum* subsp. *ossanum* essential oil (active ingredient of PAO-2) is composed mainly of sesquiterpenes, monoterpenes and oxygenated compounds; the main components of this oil were camphene, camphor, piperitone and viridiflorol (Pino et al., 2011). These compounds and some essential oils containing them were toxic to stored-product insect pests; for instance, *Sitophilus granarius* L and *Rhyzopertha dominica* F. showed very high susceptibility to camphor (Pérez et al., 2010). This monoterpenoid may be one of the components responsible for the observed biological activity of PAO-2 on *L. serricorne*.

Against *S. zeamais*, treatments that caused at least 40% mortality of adults or at least 50% reduction of the F1 emergence in relation to the untreated control were considered promissory (Silva et al., 2003).

Our results confirmed the findings of other workers that reported the Asteraceae family as a promising source of metabolites, with insecticidal activity against *S. zeamais*. Grainge and Ahmed (1988) reported the contact toxicity of the compounds present in leaves of *T. diversifolia* against *S. zeamais*. Mortality by the leaves was higher than the rest of the structures (stems and roots). These results were in agreement with those obtained in other studies, which reported substances with insecticidal activity in leaves of this plant against *Dysdercus cingulatus* (Fab.), *Plutella xylostella* L., *Spodoptera exempta* (Walker) and *Tribolium castaneum* Herbst (Grainge and Ahmed 1988). The highest value of insect mortality, reached with the powders of the leaves, could be due to the higher concentration of α-amino groups, which was made evident by the more intense color observed for this plant structure sample in the phytochemical screening (González et al., 2010). Other groups of compounds responsible for this effect could be phenols, tannins and triterpenes (González et al., 2010). The *T. diversifolia* appears among the plants from which crude preparations are recommended for insect control in different tropical regions (Isman, 2014).

No references of the effect of *L. punctatus* on *S. zeamais* were found according to the reviewed literature. The insecticide effect observed with the powders from the stems of *L. punctatus*, could be associated to the action of compounds or mixture of compounds belonging to the groups found in the phytochemical screening of this material, such as the α-amino groups, phenols, tannins, triterpenes/steroids, and alkaloids (González et al., 2011). The insecticidal effect of the raw meal of seeds of *C. ensiformis* against *Sitophilus oryzae* L. was reported by Zamora (2005), but no information was found regarding the effect of the powder of the seeds and the other studied parts of this plant against *S. zeamais*.

The Fabaceae family, represented in this research by *L. punctatus* and *C ensiformis,* comprises a wide group of species with insecticide activity. Among them, the most popular belong to the genera *Lonchocarpus*, *Derris* and *Tephrosia* (Gahukar, 2014; Isman, 2014). The substances from these genera act topically and by ingestion; besides, they are characterized by exerting their insecticide effect through the inhibition of the mitochondrial respiration (Grainge and Ahmed 1988; Koona et al., 2007). There are several species of this family with insecticidal action in which the presence of alkaloids and tannins is reported (Grainge and Ahmed 1988). According to Pérez and Iannacone (2006), the alkaloids is a group of very active compounds that block the transmission of the motor nerve, causing relaxation and flaccid paralysis of the skeletic muscle in the body of some insects, indicating that these compounds could be related to the insecticide action observed. In this family of plants, the non-protein amino acids are also reported (Udedibie, 2001) and they have a double function: defense and storage of nitrogen. Probably, the mortality produced by the seed powder of *C. ensiformis* was due to the presence of these compounds. The toxic effect is produced by their structural analogy with the essential amino acids, when they are misincorporated in the formation of enzymatic proteins or neurotransmitters (Ramos et al., 1998).

Besides, the acute insecticide effect on adults of *L. serricorne* and *S. zeamais*, it was necessary to assess the effect of the powders on the F1 emergence because of the high rate of their reproduction. The tested treatments that had the highest mortality in the adult insect of *L. serricorne* and *S. zeamais* also provoked the lowest emergence; the decrease of the adult emergence could be a method of effective control. The results obtained in the reduction of the emergence could be due to different causes; it could be related to an initial insecticidal effect and/or to deletorius physiological changes that affect reproduction or growth (Napoleão et al., 2013, Gahukar, 2014, Isman, 2014). The immediate death of the females and/or males may disrupt mating and thus the egg laying is reduced. A female may die before ovipositing the normal number of eggs, may survive treatment and become sterile or lay eggs that do not hatch. Other aspects that could affect reproduction are associated with the behavior. These active compounds could hamper the movement and encounter of the male with the female by occupying the space between the grains affecting the mating, or that the female by finding the grain covered with the powder does not receive the stimulus necessary for egg laying, a situation that could be caused by the allomones of the plant that are released to the environment where the grains are found (Shenk and Kogan 2003). Also, the death of the insects in the immature stages hampers the insect emergence. Other studies are required to determine the actual factors involved in the reduction of the F1 progeny by the evaluated products.

Additionally, all the botanical derivatives, tested on *L. serricorne* or *S. zeamais*, showed a repellent effect, except *T. diversifolia* roots. The results suggested that PAO-1 would have greater application in pest control for its effect on insect behavior to prevent grain infestation. The repellent effect could be determined by the presence of secondary metabolites, volatile substances that may be present in the plant parts studied (Regnault-Roger et al., 2004). When the insects detect these substances, they cause an effect on their behavior and evoke them to move away from product (Pérez et al., 2007). The attractive action of *T. diversifolia* root powder could be explained by the diversity of groups of secondary metabolites detected in the different tissues of this species under the conditions in which it was collected (González et al., 2010).

No available information was found about the repellent effect of the studied plants against *S. zeamais* or *L. serricorne*. But it is known that farmers sow *C. ensiformis* plants as intercrop and barrier to avoid the attack of pests (Arim et al., 2006; González et al., 2009). Also, *T. diversifolia* repellent action on the herbivorous *Atta* sp has been reported (Medina et al., 2009).

Further work is in progress to isolate and identify the insecticidal and repellent constituents of the plants studied. The sustainable use of these plants in the storage pest management is possible; they are cultivated or abundant wild species in Cuba. However, their practical application will require subsequent environmental and economic assessment. These products have potential for small-scale treatments of grains for protection against both insects. The *P. aduncum* subsp. *ossanum*-based products may also be used to control *L. serricorne* infestation in tobacco. The identification of local candidates to develop effective and safe pesticides offers new alternatives to the Cuban agriculture in the control of storage pests.

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# Activity of two deltamethrin formulations on different surfaces against rice weevil, *Sitophilus oryzae* (L.)

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## Abstract

Several methods are used to control stored product insects. The spraying of empty structures with insecticides, prior to the introduction of produce, is an important method for preventing development of insects. It is known that insecticide activity varies according to the various sprayed surfaces. In this study, the activity of deltamethrin was examined on concrete and plastic surfaces. Deltamethrin is a synthetic pyrethroid, active by contact against a variety of insects; applied in Israel in two formulations: KESHET 2.5% EC and BUNGY 1.5% SC (ADAMA Makhteshim Ltd.). Adults of the rice weevil, *Sitophilus oryzae* (L.), served as target insect in all experiments. The research was carried out in plastic Petri dishes and in Petri dishes with layer of concrete. Deltamethrin (KESHET 2.5% EC) was applied in water solution in doses of 0.02, 0.1, 0.5 g/m$^2$. Without concrete, complete mortality of *S. oryzae* was obtained at a concentration of 0.02 g/m$^2$, whereas in concrete plates, no mortality was found in all 3 concentrations. In contrast, deltamethrin (BUNGY 1.5% SC) in doses of 0.1 g/m$^2$, caused 100% mortality with and without concrete layer. The same results were found in the commercial warehouse. No difference in efficiency was found between the spraying methods: airbrush (Sparmax DH-125) or dripping by pipette. The results show that the efficacy of warehouse spraying by deltamethrin depends on its formulation.

**Keywords:** deltamethrin, stored product insects, treated surfaces, concrete, suspension, emulsion.

## 1. Introduction

The control of stored product insects, which cause serious damage to stored agricultural produce, is achieved by a combination of several methods. The use of protectants is an important and widespread method for preventing the development of stored product insects as an integral part of pest control practice. Protectants are applied by spraying incoming grains and empty warehouses, prior to produce introduction; in order to disinfest insect population on surface and cracks. Using residual materials can reduce and slow insects' infestation and reduce or eliminate the need for further control treatments. Surface treatments are applied as liquid contact insecticides on different types of surfaces (Arthur and Subramanyam, 2012). Floor and walls of grain warehouses are usually made of concrete. The surface of concrete is porous and alkaline, so when the insecticides are applied to concrete, hydrolysis and rapid degradation occur (Arthur, 1994; Jain and