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Effect of extract of *Argemone mexicana* L. seeds on the mortality, fecundity and emergence of *Callosobruchus maculatus* (F.) for the protection of the stored grains

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Abstract
Cowpea beetle, *Callosobruchus maculates* (Fabricius) (Coleoptera: Chrysomelidae: Bruchidae), is the most notorious store grain pest. Pulses constitute the major source of protein in most of the developing countries including India. The stored grain pests are difficult to control because of chemical insecticides if used causes health hazards to us. Adult *C. maculates* were exposed to 4, 8, 12 and 16ml concentration of extract/Kg grains of mung bean (*Phaseolus aureus* (Roxb) and were homogenized separately.

After the treatment with acetone extract the mortality was recorded at 4ml concentration of extract per kg grains of mung bean, was observed i.e. 50%. The number of eggs laid were 115±5.00, grains infected were 109.33±3.21 and adult emerged were 98±3.00 respectively while at above 12ml concentration of extract per kg grains of mung bean, the adult mortality was high i.e. 100%. There was no infestation and emergence of adults recorded. In chloroform and ethanol extracts of *Argemone mexicana* L seed the adult mortality was high 100%, there was no infestation and emergence of adult recorded at all concentrations per kg grain of mung bean. Methanol extract had very poor toxic effect and low inhibitory effect on the reproductive activity and hence, large number of the adults emerged out, they can damage the *P. aureus* grains severely in the forthcoming generations.

As the concentration increases, significant reduction in adult emergence takes place. Seed extract of *A. mexicana* L. in acetone, chloroform and ethanol solvent shows highest mortality, no infestation and no adult emergence as compared with control while based on these findings, it can be used to control the infestation of stored grain pest *C. maculatus* F.

Keywords: Bruchid, homogenized, infestation, seed extract, *Phaseolus aureus*

Introduction

Insects are the most numerous and successful animals on earth and well known for their beneficial and harmful effects in agriculture. They cause heavy losses to stored grains throughout the world and their impacts are more devastating in developing countries (Ekeh *et al*., 2013) [19].

Argemone mexicana L. is an erect prickly annual exotic weed with yellow flower and latex. It belongs to the class Magnoliopsida and family Papaveraceae. It is a native of tropical America and now widely naturalized in tropics. The common mane of this species is Mexican Poppy, Mexican Prickly Poppy or Cardosanto. It is shrub, 45-75 cm. high, prickly, leaves 2.5-4 cm. long, sessile, sinuate-lobbed, blotched with white, the lobes tipped with slender yellow spines; flowers pale-yellow, seeds small and black (Boston, 1979) [12]. Chemical investigations of this plant have revealed the presence of alkaloids (Hussain *et al*., 1983; Nakkady *et al*., 1988) [24, 36], amino acids (Dinda *et al*., 1986) [16], phenolics (Harbone *et al*., 1983) [23] and fatty acids (Gunstone *et al*., 1977) [22]. *A. mexicana* L. is considered as an important medicinal plant in India; the yellow juice, which exudes when the plant is injured, has long been used in India as traditional medicine for dropsy, jaundice, ophthalmia, scabies and cutaneous affections (Chopra *et al*., 1956; Ambasta, 1986; Sharma *et al*., 2012) [14, 5, 53]. Different parts of this plant are used in chronic skin diseases, and also as emetic, expectorant, demulcent and diuretic; the seeds and seed oil are employed as a remedy for dysentery, ulcers, asthma and other intestinal affections (Chopra *et al*., 1956; Bose *et al*., 1963; Ambasta, 1986; Prajapati *et al*., 2003; Savithramma *et al*., 2007) [14, 11, 5, 44, 50]. Leaves and seeds are also reported to find application in maintaining normal blood circulation and cholesterol level in human body (Albuquerque *et al*., 2007) [3].

The plant contains many alkaloids (Sangwan and Malik, 1998; Yu Chwen, *et al*., 2003) [49, 64] and was found to possess larvicidal and growth inhibiting activity against the second instar larvae of *Aedes aegypti* Linnaeus (Sakthivadivel and Thilagavathy, 2003) [48].

Pulses are the second most important group of crops worldwide. About 870 million people are undernourished because of inadequate intake of proteins, vitamins and minerals in their diets (FAO, 2012) [21]. Pulses are excellent sources of proteins (20-40%), carbohydrates (50-60%) and are good sources of calcium and iron.

Pulse bruchids, the *Callosobruchus* species are among the most destructive storage grain pests and known to occur throughout the world on different types of pulses. These are considered as notorious pests of green gram, chick pea, black gram, peas, cowpea, lentil and pigeon pea (Aslam *et al*. 2002) [34].

Cowpea beetle, *Callosobruchus maculates* F. (Coleoptera: Chrysomelidae, Bruchinae) is a major pest that causes serious damage (Sharma, 1989) [54] on stored pulse grains. It is cosmopolitan in distribution. *C. maculatus* F. has caused enormous weight loss, reduced viability and reduced commercial value of cowpea seeds (Adedire *et al*., 2004; Emeasor *et al*., 2005) [2]. Up to 100% infestation of cowpea can occur after three to six months storage (Maina *et al*., 2011) [32], resulting in about 60% weight loss (Umeozor, 2005) [60].

The female lay their eggs on the seeds in the storage; larvae feeding on the cotyledons causes substantial quantitative and qualitative losses (Jackai and Daoust 1986; Ogunwolu and Odunlami 1996; Pascual-Villalobos and Ballesta-Acosta 2003) [26, 40, 43]. Over 90% of the insect damage to cowpea seeds is caused by *C. maculatus* F. (Caswell 1981) [13].

The use of synthetic insecticides still remains the most effective means of controlling field and stored product insect pests (Lale, 2002) [29], but their use has several drawbacks such as high cost, inconsistent supplies, hazards to man and the environment (Ofuya, 2003) [39]. It also causes the genetic variations in plant populations, reduction of beneficial species, damage to the water bodies, poisoning of food and health problems such as cancer.

In order to control beetle without using conventional chemicals, Arannilewa *et al*., (2006) [6] and Emeasor *et al*., (2007) [20] suggested the use of plant materials either singly or in mixtures to protect grains against pest damage during storage.

The total world production of biopesticides is over 3,000 tons/yr, which is increasing at a rapid rate. Biopesticides are biochemical pesticides that are naturally occurring substances that control pests by nontoxic mechanisms. Many groups of phytochemicals such as steroids, alkaloids, terpenoids, phenolics and essential oils from more than 2000 plant species have been reported previously for their insecticidal activities.

Much research has been conducted on the use of natural products, especially on plant extracts having insecticidal properties and whose undesirable effects have not been reported in humans (Isman, 2000) [25]. These natural compounds from plants such as essential oils could be efficient alternatives to conventional fumigants because of their low toxicity to mammals, high fumigant activity due to their high volatility, fast degradability properties and regional availability (Rajendran and Sriranjini 2008; Ayvaz *et al*. 2010) [46, 8]. Most of these substances were tested against insect attacking stored products in order to establish new control practices with low persistence in the environment and potential for commercial application (Liu *et al*. 2005) [30]. Thus the essential oils extracted from plants have been widely used in the fight against pests of grain stocks (Regnault and Hamaoui, 1997; Prates *et al*., 1998; Tapondjou *et al*., 2002; Tapondjou *et al*., 2003; Ngamo *et al*., 2007a; Nerio *et al*., 2010) [47, 45, 57, 58, 38, 37].

In the present study, seeds of *A. mexicana* L. have been selected as one of the safer substitutes to control the stored pest cowpea beetle, *C. maculatus* F.

Materials and methods

Insect culture

Infected pulses were obtained from the local market and the adult beetles were identified from Agriculture College, Aurangabad, Maharashtra, India. Parental stocks of *C. maculates* F. were reared at 28±2 ºC and 70% relative humidity (RH) on the grains of *Phaseolus aureus* (Roxb). Ten males and ten females (10 pair) were released in the plastic bottle containing 500g of *P. aureus* grains and were allowed to grow. The mouths of the jar were tied with moist muslin cloth to maintain the required humidity for the survival of the pest. The adult females laid eggs on the grains and then died. Dead beetles were removed and the culture was maintained at 28±2 ºC and 70% relative humidity (RH) on the grains of *P. aureus.* After about 28 days, the young adults began to emerge out from the grains. The adults hatched were used for the experimentation and some were released in the healthy mung bean again to maintain the stock culture.

Collection of plant material and extraction

Seeds of *A. mexicana* L. were collected from the field near Aurangabad, Maharashtra, India and were identified by department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India. These seeds were dried in the shade and then in oven. The dried seeds were powdered in the grinder. The powder was packed in filter paper and extract was extracted in Soxhlet apparatus in 1:10 ratio i.e. 20 gm powder in 200 ml solvent. After eight hours of continuous extraction the final extract was kept open to evaporate the solvent and remaining as stock solution extract was stored at 4 ºC in a refrigerator until use. Extracts were extracted in chloroform, methanol, ethanol and acetone were stored after evaporation of solvent in refrigerator.

Experimental set up

The seeds of *A. mexicana* L. extract were separately mixed with 25g *P. aureus* R. (mung bean) at 4, 8, 12 and 16ml concentration of extract per kg grains of mung bean and were placed into 50 g capacity plastic bottles then 5 pairs of freshly emerged *C. maculates* F. adults were placed into the plastic bottles and covered with muslin cloth and rubber band to prevent escapes. The whole experiment was repeated thrice.

The mortality, number of eggs laid, number of grains infected and number of adults emerged of *C. maculatus* F. adults was recorded after every 24 h and percentage (%) data collected was analyzed.

Results

Increased adult mortality and low fecundity was observed with the increase in concentration of extracts of *A. mexicana*

L. (Table no. 1) and it is graphically represented in the figure 1. In *A. mexicana* L. seed extract in chloroform at 4ml concentration of extract per kg grains of mung bean, 8ml concentration of extract per kg grains of mung bean, 12ml concentration of extract per kg grains of mung bean and 16ml concentration of extract per kg grains of mung bean 100% adult mortality and egg laying was not observed against the adult was recorded at 96 hours while no adult emergence was recorded in chloroform extract.

In ethanol extract at 4ml concentration of extract per kg grains of mung bean 90% while 100% adult mortality was recorded at 8ml concentration of extract per kg grains of mung bean, 12ml concentration of extract per kg grains of mung bean and 16ml concentration of extract per kg grains of mung bean. Egg laying was recorded at 4ml concentration of extract per kg grains of mung bean(1.66± 2.08) while at 8ml concentration of extract per kg grains of mung bean, 12ml concentration of extract per kg grains of mung bean and 16ml concentration of extract per kg grains of mung bean the egg laying and adult emergence was not recorded.

The mortality was recorded in acetone extract at 4ml concentration of extract per kg grains of mung bean were recorded i.e. 50%. The number of eggs laid were 115±5.00, grains infected were 109.33±3.21 and adult emerged were 98±3.00 respectively. At 8ml concentration of extract per kg grains of mung bean the mortality was 100%, number of eggs laid were 4±1.00, grains infected were 1.33±0.57 and adult emergence were 1±0.00 respectively while at above 12ml concentration of extract per kg grains of mung bean, the mortality was high 100%. There was no infestation and emergence of adults recorded. The number of eggs laid was much reduced in treated samples in comparison with untreated (control). Progeny emergence in each treatment was significantly low compared to the number produced in the control. Methanol extract had very poor toxic effect and low inhibitory effect on the reproductive activity and hence, large number of the adults emerged out, they can damage the *P. aureus* grains severely in the forthcoming generations.

Chloroform, acetone and ethanol seed extract of *A. mexicana* L. were most effective extracts which affected significantly, the egg laying of the adult and further development was not found while the development of the adult was observed in methanol extract.

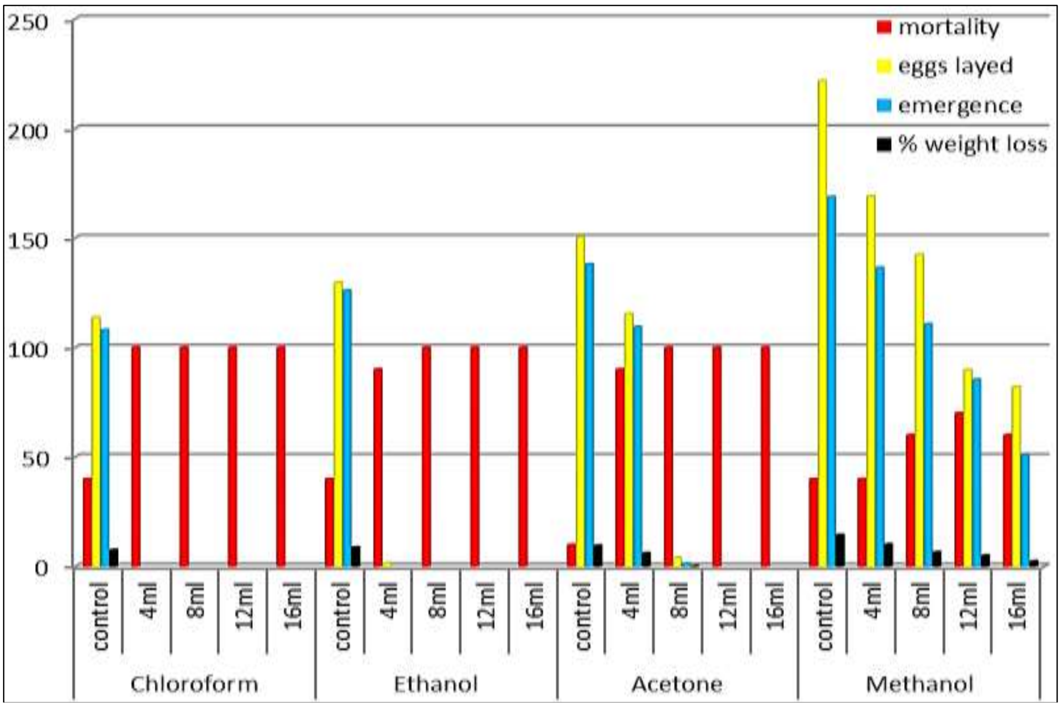

Fig 1: Efficacy of seed extract of *Argemone mexicana* in chloroform, ethanol, acetone and methanol solvent against adult mortality, fecundity and emergence of *Callosobruchus maculatus*

Table 1: Effect of extract of *Argemone mexicana* seeds on the mortality, fecundity and development of *Callosobruchus maculatus*

Solvent	Concentration Ml extract/Kg Mung bean	Mortality (in hrs) %				Eggs layed	Adult Emergence	Percent weight loss in grains %
		24	48	72	96			
Chloroform	Control	-	-	10	50	113.66 ± 7.09	108 ± 3.46	7.57
	4	-	10	70	100	0	0	0
	8	-	40	100	-	0	0	0
	12	-	40	100	-	0	0	0
	16	70	100	-	-	0	0	0
Ethanol	Control	-	-	10	40	129.66 ± 21.22	126 ± 17.34	8.84
	4	-	10	70	90	1.66 ± 2.08	0	0
	8	10	30	50	100	0	0	0
	12	30	30	100	-	0	0	0
	16	20	50	100	-	0	0	0
Acetone	Control	-	-	-	10	150.33 ±11.93	138 ± 15.87	9.60
	4	-	20	40	80	115.33 ±7.63	109.33 ± 10.96	6.08

	8	20	70	100	-	4 ± 1	1.33 ± 2.30	0.1
	12	30	30	100	-	0	0	0
	16	20	20	100	-	0	0	0
Methanol	Control	-	10	10	40	221.66 ± 58.73	168.66 ± 3.51	14.42
	4	-	-	20	40	169 ± 14.42	136.33 ± 29.50	10.16
	8	-	10	20	60	142.33 ± 23.18	110.66 ± 13.05	6.53
	12	-	10	20	70	89.66 ± 25.38	85.33 ± 31.13	5.04
	16	10	10	30	60	82 ± 7.81	51 ± 14.17	2.36

Discussion

The present study showed the effect of different dose levels of *A. mexicana* L. extracts in chloroform, ethanol and acetone caused high adult mortality, low fecundity of *C. maculatus* F. as compared with untreated.

Boateng and Kusi, (2008) [10] investigated toxicity of Jatropha seed oil to *C. maculates* F. and reported that Jatropha seed oil was highly toxic to the eggs of *C. maculatus* F. at all dosage levels compared with other pre-adult stages.

According to Yahaya, (2002) [63] the oil and powder of *Piper guineense* Schumach significantly reduced the egg laying capacity of adult *C. maculatus* F. weevils, as well as their survival on cowpea grains.

Vegetable oils have been noted to reduce oviposition by *C. maculates* F. and Neem oil extract to be most effective in hampering oviposition (Nailk and Dumbre, 1984) [35]. Khaire *et al*., (1993) [28] reported that seeds treated with Neem oil had a repellent action against egg laying activities of adult *Callosobruchus chinensis* Linnaeus beetles for up to 100 days after treatment. Also according to Yadav, (1985) Neem seed oil has been reported to prevent oviposition by *C. maculates* F. on green grains as compared to *C. chinensis* L. and *Callosobruchus analis* Fabricius.

Minimum egg laying by *C. maculates* F. have earlier been recorded when cowpea seeds were treated with dry ginger root powder, pulverised dried Neem fruit of cashew nut oil (Echendu *et al*., 1998) [18].

Shukla *et al*., (1988) [55] reported that oils of coconut, sesame, rape, soyabean, groundnut, mustard, palm and maize and dalda were found to be effective in reducing the number of eggs laid by *C. maculatus* F. on cowpea seeds. When chickpea grains were treated with the oils of groundnut, coconut, mustard, sesamum, soyabean significantly reduced oviposition by *C. chinensis* L. have also been noted (Singhal *et al*., 1990) [56]; the treatments of karanj oil and castor oil effectively brought down the number of eggs laid by the bruchid *C. chinensis* has also been suggested (Babu *et al*., 1989) [9]; the total number of eggs laid on the seeds treated with Neem oil was significantly lower than in the untreated seeds (Das, 1986) [15]; significant reductions in oviposition by *C. maculates* F. when treated with groundnut oil and palm oil have also been indicated by earlier workers (Uvah *et al*., 1992) [61].

The present result agrees with the observations made by other researchers (Ohazurike *et al*., 2003; Abulude *et al*., 2007; Udo, 2011) [41, 1, 59] mixing of groundnut oil with *Vigna unguiculata* Linnaeus probably resulted in thin smooth oil coating on treated grains and this may had limited contact between the grains and the weevils. Death of the weevils may have resulted from interference with normal respiratory activity.

The mode of action of essential oils is yet to be confirmed, but it seems that death of the insects may be due to suffocation and inhibition of different biosynthetic processes of the insect's metabolism (Don-Pedro, 1989) [17]. Park *et al*., (2002) [42] reported that some constituents of many plants such as linalool, terpineol, carvacrol and myrcene have insecticidal effects on some stored products pests.

According to Kangade and Zambare, (2013) [27] the extract of leaves of *A. mexicana* L. in methanol was useful to control the infestation of *Corcyra cephalonica* Santonin rice.

The effect of cotton seed oil on reducing the fecundity among female *C. maculatus* F. indicates the presence of certain compounds in the oil which might be responsible for this activity. When applied on beans, the crude oils of cotton seed, soya beans and coconut palm were very effective in reducing the oviposition of *C. maculatus* F. (Schoonhoven, 1978; Mahfuz *et al*., 2007) [51, 31].

The ingestion of maize leaves that were treated with 30% *Argemone ochroleuca* Linnaeus extract caused significant direct mortality (31.01 ± 2.18%) in *Spodoptera frugiperda* Smith 3rd instar larvae until just before pupation compared with controls (10.25 ± 2.56%) (Martínez *et al*. 2017) [33]. According to Vetal and Pardeshi (2019) [62] the toxicity of ethanol and aqueous leaf extract of *A. mexicana* L. was tested against III instar larvae of *Spodoptera litura* Fabricius. In their study mortality increased with increase in concentration at all the doses up to 96 h of exposure. Sharma *et al*., (2016) [52] evaluated the effect of *A. mexicana* L. leaves extract of different solvents on gut of *Heliothis armigera* Hubner and after 24 and 96 h of treatment *Heliothis armigera* (Hub.) showed severity of the damage of epithelial lining and epithelial cells with vacuoles at certain places. Ashwini *et al.*, (2017) [7] found that the toxicity bioassay of *A. mexicana* L. extracts caused greater mortality on third instars larvae (LD50 = 5.33 mg-1) than *C. inerme* Linnaeus (LD50 = 7.26 mg-1).

Conclusions

The results obtained from this assay revealed that all extracts of *A. mexicana* L. seeds have shown varying levels of insecticidal property against *C. maculates* F. The importance of *A. mexicana L.* for the control of stored grain pest such as *C. maculates* F. has been proved. This can be used as an alternative for the chemical methods.

Abbreviations

% - Percent.
Ml - Milliliter.
Kg - Kilogram.
g - Gram.

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