Kurdistan Region Government-Iraq Ministry of High Education and Scientific University of Salahaddin-Erbil College of Agricultural Engineering Sciences Plant Protection Department



Impact of Some plants on the management of pulse beetle *Callosobruchus maculatus* (Coleoptera: Chrysomellidae)

Prepared by:

Asma Abdulqadir Rahman

Supervised by:

Dr.Shatha .H.Ahmed

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1. Introduction

Legumes are the second most important group of crops worldwide. Globally, 840 million people are undernourished mainly on account of inadequate intake of proteins, vitamins and minerals in their diets, pulses are excellent sources of proteins (20-40%), carbohydrates (50-60%) and are fairly good sources of thiamin, niacin, calcium, and iron (CABI, 2007).

Insects have been causing tremendous losses not only to the crops growing in fields but also to postharvest commodities during storage. Significant losses in grains, both quantitative and qualitative, occur during storage and the damage caused by insects may amount to 5-10% in the temperate and 20 - 30% in the tropical zone.

The worldwide losses in storage due to insects and rodents have been estimated by FAO to be about 20%, the figures ranging from 10% in Europe and North America to 30% in Africa and Asia (Nakakita, 1998).

Among insect pests of stored legumes, *Callosobruchus maculatus* (F., 1775) is a major pest of economically important leguminous grains such as cowpeas, lentils, green gram and ranked as the most important with seed loss as high as 60-100% having been reported. Chemical control using synthetic insecticides is usually advocated and have undesirable side effects including human and animal health concerns, development of insecticide resistance and environmental contamination (Khan 2021, Park, et al., 2003).

Yallappa *et al.* (2012) observed that newer insecticides will have to meet entirely different standards in developing new and alternate pesticides.

They must be pest specific, non-phytotoxic, non-toxic to mammals, ecofriendly, less prone to pesticide resistance, less expensive, and locally available. Requirements which had led to re-

examination of the century-old practices of protecting stored products using plant-derivatives (Sahayaraj, 2008).

Plants based pesticides are chemicals isolated from various plants which could be used to control pests in a non-toxic mechanism. Several plants have certain bioactive compounds which could be used as alternatives to hazardous synthetic pesticides for pest control. (Akbar, et al., 2022). The overall objective of this study is to

assess the insecticidal property of some botanicals usually used, tobacco leaf, basil leaf and dry powdered pepper. Also, the possible combinations of these botanicals for optimum, and effective results

2. Literature review

2.1 Classification of Callosobruchus maculatus

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Arthropoda

Subphylum: Uniramia

Class: Insecta

Order: Coleoptera

Family: Chrysomelidae

Genus: Callosobruchus

Species: Callosobruchus maculatus (F., 1775).

2.2 Distribution of C. maculatus

The two most widespread species of bruchid beetle are *C. maculatus* and *C. chinensis*, which are distributed throughout the tropics and sub-tropics. *C. maculatus* originated in Africa where it remains dominant (CABI,2022).

Fig.1 Distribution map of C. maculatus



www.cabi.com,2022

Table 1. distribution of C. maculatus

Continent/Country/Region	Distribution	Last Reported	Origin	First Reported	Invasive	Reference	Notes
Africa							
Benin	Present					Kossou et al. (2001) 🛛 🗸	
Burkina Faso	Present, Widespread					Ouedraogo et al. (1996)	
Cameroon	Present					Ngamo et al. (2007)	
Egypt	Present, Widespread					Hammad (1978) 🗸 🗸	
Eswatini	Present, Widespread					Haines (1981)	
Ethiopia	Present					CABI (Undated)	
Ghana	Present, Widespread					Haines (1981)	
Kenya	Present, Widespread					Khamala (1978) 🛛 🗸	
Malawi	Present, Widespread					Haines (1981)	

Asia					
Bangladesh	Present, Widespread			Kabir (1978)	
China	Present	Introduced		Seebens et al. (2017)	First reported: 1960s
India	Present, Widespread			Vir and Jindal (1981) 🗸	
Iran	Present			Taheri (1996)	
Israel	Present	Introduced	1962	Seebens et al. (2017)	
Japan	Present	Introduced	1950	Seebens et al. (2017)	
Kuwait	Present, Widespread			Haines (1981)	
Pakistan	Present, Widespread			Sulehrie et al. (1998)	
Sri Lanka	Present, Widespread			Haines (1981)	
Taiwan	Present, Widespread			Rose et al. (1978)	
Turkey	Present			Mergen and Çaghacek [~] atay (1996)	
Yemen	Present, Widespread			Haines (1981)	
Europe					
Austria	Present	Introduced	1983	Seebens et al. (2017)	

2.3 Host range of C. maculatus

The cowpea seed beetle, *C. maculatus* is the most serious insect pest attacking grain legumes in storage throughout the tropics and subtropics. While it has been reported to attack at least 21 species of legumes, its preferred hosts include *Mill* sp, *Glycine max* (L.) *Cajanus cajan* (L.) Merr., *Phaseolus* spp and *Vigna unguiculata* (L.) Walp. (Khan,2021, CABI,2022).

Pulse grains are usually vulnerable to be attacked by many stored grain insect pests including *Callosobruchus* species such as *C. analis*, *C. chinensis* and *C. maculatus*. Among them *C. maculatus* causes heavy losses to all pulses in storage conditions. It infests almost all kinds of whole pulses such as mung gram or green gram, mash gram, chickpea, kidney beans, lentils, pigeon pea, pea nut, cowpea, dry Peas and adzuki beans. (Majeed *et al.*, 2016, Hajam and Kumar, 2022, Perzada *et al.*, 2022)

The pulse beetle *C*. sp. is a major pest of economically important leguminous grains such as cowpeas, lentils, green gram, and black gram (Park *et al.*, 2003).

2.4 Distribution table of C. maculatus

The distribution in this summary table is based on all the information available to CABI,2022. When several references are cited, they may give conflicting information on the status.

Table 2. Host range of C. maculatus

Acacia nilotica (gum arabic tree)	Main	
Anacardium occidentale (cashew nut)	Unknown	Agboton et al. (2014)
Astragalus cicer (Cicer milkvetch)	Other	
Cajanus cajan (pigeon pea)	Main	Anand et al. (2015)
Cicer arietinum (chickpea)	Other	Anand et al. (2015)
Fabaceae (leguminous plants)	Main	
Glycine max (soyabean)	Main	
Lablab purpureus (hyacinth bean)	Other	
Lathyrus sativus (grass pea)	Other	
Lens culinaris subsp. culinaris (lentil)	Main	
Lupinus albus (white lupine)	Other	
Phaseolus (beans)	Main	
Phaseolus acutifolius (tepary bean)	Other	
Pisum sativum (pea)	Other	
stored products (dried stored products)	Main	
Vigna (cowpea)	Other	
Vigna aconitifolia (moth bean)	Unknown	Anand et al. (2015)
Vigna angularis (adzuki bean)	Other	
Vigna mungo (black gram)	Other	
Vigna radiata (mung bean)	Main	
Vigna umbellata (rice bean)	Other	
Vigna unguiculata (cowpea)	Main	
Vigna vexillata (wild sweetpea)	Other	

CABI,2022.

2.5 Symptoms of attacked grains

In the early stages of attack the only symptoms are the presence of eggs cemented to the surface of the pulses. As development occurs entirely within the seed, the immature stages are not normally seen. The adults emerge through windows in the grain, leaving round holes that are the main evidence of damage.

2.6 Impact of C. maculatus

Callosobruchus spp. are important pests of pulses. Infestation may start in the pods before harvest and carry over into storage where substantial losses may occur. In Nigeria, it has been estimated that 3% of the annual production in 1961/62 was lost due to attack by *C. maculatus*. Levels of infestation in storage are strongly influenced by the type of storage structure employed and the variety of seed Storage structures that maintain elevated levels of moisture in seeds are more prone to high levels of infestation. Temperature of storage also influences levels of infestations in local stores. The values of dried pulses are strongly influenced by levels of bruchid infestation in local markets, particularly in Sub-Saharan Africa. (Singh 1999, Ojimelukwe et al., 1999).).

2.7 Life cycle of C. maculatus

Mating took place within 40 to 60 minutes of adult emergence where male had a stimulant role in copulation and its duration 30-40 seconds. Sometimes male and female showed courtship behavior before mating and the male always ran after the female.

The eggs were translucent in color, glued with seed coats just after oviposition and became whitish in the next day. The adult weevils and eggs are found on grain surfaces, while larvae and pupae live

inside the grains. The eggs are cemented to the surface of pulses and are smooth, domed structures with oval, flat bases. Eggs of *C. maculatus* are oviposited on the surface of leguminous seeds and enclose in 6-7 days. The duration of the larval, pre-pupal and pupal stages is 32, 3.5 and 4 days respectively at 230C and 75% R.H. (Moreno et al., 2000). The larvae bore into the grains by making circular holes where they feed on endosperm. The duration of first, second, third and fourth larval instars are 6, 3, 4 and 4 days whereas pre-pupal and pupal are 1 and 5 days followed by 1 day of emergence. The first instar larvae are small and second and third are apparently similar except the growth and development. However, before pupation, the fourth instar larva started to change its morphology completely and ongoing development turned it to adult individual. The average longevity

of virgin male and female was estimated as 6.5±1.5 days and 10.5±3.5 days ((Ahmady *et al.*, 2016, Rahman, et al. 2022).



Fig.2 Life cycle of C. maulatus



Fig. 3 Egg of C. maculatus



Fig.4 larvae of C.maculatus



Fig.5 pupa of C. maculatus

2.7.1 Adult

The adult males were smaller, brownish, with obtuse abdomen, pectinate antenna (larger) and hypermobility whereas the females were larger, blackish, pointed abdomen, serrate antenna (shorter) *C. maculatus* adults are 2.0-3.5 mm long. Females often have strong markings on the elytra consisting of two large lateral dark patches mid-way along the elytra and smaller patches at the anterior and posterior ends, leaving a paler brown cross-shaped area covering the rest. The males are much less distinctly marked. In common with other species of *Callosobruchus, C. maculatus* has a pair of distinct ridges (inner and outer) on the ventral side of each hind femur, and each ridge bears a tooth near the apical end. The inner tooth is triangular, and equal to (or slightly longer than) the outer tooth. (Mbata et al., 2013, Rahman et al., 2022,).



Fig. 6 Female of C.maculatus



Fig.7 male of C.maculatus

2.8 Damage of Callosobruchus spp

There are multiple reports of damage caused by *Callosobruchus*, both in the field and on stored grains,74.67% damage on pigeon pea (*Cajanus cajan*) variety ICP 10531 was recorded in Tamil Nadu in 2008 and 2009. In India, *C. chinensis* damage of stored chickpea (*Cicer arietinum*) peaked at 13.75% during January and February recorded seed damage of 96.15% for cowpea, 88.44% for mung bean (Vigna radiata) and 74.22% for moth bean (*Vigna conitifolia*) The Southern Cowpea Beetle *C. maculatus* (F.) is one of the most widespread insect pests of stored legumes, causing a considerable loss during storage, decreasing the net weight of the crops, and resulting in reduced the quality of the crops. *C. maculatus* is a serious economic pest of stored legumes and seed infestation starts in the field. Feeding injury by the developing larvae reduces the amounts of carbohydrates and proteins of the grain, leading to the degradation of the nutritional quality and forcing the growers to sell their

commodity with low economic value after harvest. It is estimated that the economic losses caused due to *C.maculatus* infestation in stored grain legumes are 35%, 7-13%, and 73% in Central America, South America, and Kenya, respectively. Legume seeds stored for six months can experience 70% seed infestation and about 30% yield loss, leaving them unacceptable for human consumption. However, there is not enough information about the economic injury level (EIL) of *C. maculatus* under storage conditions (Murdock et al. 2003, Sharma et al., 2013, Hamdi et al. 2017, Jassim et. al., 2023).

2.9 Plant materials as biopesticides agent (Botanical pesticides)

Natural plant products have gained a lot of importance in recent years with researchers as natural sources of new insecticides. It is estimated that more than 6,500 species of plants have been examined

for anti-insect properties. Of these, more than 2,500 species belonging to 235 families have shown a bio pesticide activity (Castillo et al., 2010, Walia et al., 2012, Pavela, 2016). Families with plants that have different chemical groups for insect control had shown in table (4).

Family	Plants	Active molecules	Activity type
Annonaceae	Annona muricata L. Annona squamosa L. Annona cherimola Miller	Acetogenins, alkaloids, isoquinoline	Synergistic, insecticide, fungicide, citotoxic, antiparasitic
Liliaceae	Allium sativum L. Allium fistulosum L.	Disulfide, thiosulfinate, thiosulfonate	Insecticide, acaricide, nematicide, herbicide, fungicide, bactericide
Solanaceae	Nicotiana tabacum L. Capsicum frutescens L.	Alkaloids (atropine, nicotine, solanine)	Toxic, deterrent, non-persistent contact insecticide
Meliaceae	Azadirachta indica A.Juss. Melia azedarach L.	Limonoids terpenoids	Insecticide
Piperaceae	Piper nigrum L.	Lignans	Synergistic, insecticide

	Table.	3	Examples	s of	the most	common	plants	with	activ	vity	foe	insect	contro
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Source: modified from Tello et al. (2010).

The cowpea beetle, *C. maculatus* (F.) is associated with cowpea storage, where it can attack the whole cowpea grains. The use of plant materials in pest control could become important supplements or alternatives to imported synthetic pesticides. *C. maculatus* (F.) attacking *Vigna* species was also tested against several oils. It is, therefore, important that appropriate technology is developed to promote a direct preparation of traditional pesticides at the farm level for resource poor farmers who have no access to commercial pesticides or cannot afford them. Biopesticides are pesticides which come from plants as basic ingredients, living materials or organic matterials. The content of chemicals in plants shows bioactivity in insects, such as repellent, antifeedant, insect growth regulators, and oviposition deterrent and oviposition deterrent. (dos Santos et. al 2009, Listiyani, 2012). Manju and Shanthi (2019). Evaluated the mortality % and oviposition of *C. maculatus* treated with twelve botanical plants, *Ipomea* sp., *Ocimum sanctum* (L.), *Pongamia pinnata* (L.), *Vitex negundo* (L.) *Adhatoda* sp. (L.), *Zingiber officinale* (L.), *Acorus calamus* (L.), *Allium sativum* (L.), *Curcuma longa* (L.), *Capsicum*

annum (L.), Piper nigrum (L.) and neem seed kernel powder against the adults of pulse beetle *C. maculatus*. Idoko and Ileke (2020) studied the insecticidal activities of essential oils from seeds of five botanicals *Tetrapleura tetraptera*, *Annona muricata*, and *Aframomum melegueta* and leaves of *Eucalyptus globulus* and *Ficus exasperata* were evaluated as biopesticides against the storage pest of cowpea *C. maculatus* (Fabricius).

Iledun and Chubiyojo (2021) studied the evaluation of five plant materials on the control of cowpea weevil (C. *maculatus*), neem leaf (*Azadirachta indica*), dry pepper (*Capsicum annum*), castor leaf

(*Ricinus communis*), Moringa leaf (*Moringa oleifera*) and tobacco leaf (*Nicotiana tabacum*). The effectiveness of aqueous and ethanolic extracts of the Oleander Nerium oleander, Basil Ocimum basilicum, Chinaberry Melia azedarach, and Natgrass Cyperus rotundus leaves on chickpea Cicer arietinum L. were studied on some biological sides of the cowpea beetle C. maculatus (Ibrahim,2023). And so on.

2.10 Description of the plants

2.10.1 Ocimum bacillicum L. (Basil)

Ocimum species are herbal plants that are available in Indonesia. they are native to tropical areas such as southern Asia, Africa, and India. *Ocimum* comes from the (Lamiaceae family) which has about 50 to 150 species. Due to its pharmacological effects, this plant has been widely used traditionally for the treatment of headaches, coughs, diarrhea, constipation, warts, and kidney damage.

These properties come from the secondary metabolite components that are abundant in *Ocimum* plants such as steroids, tannins, alkaloids, flavonoids, and phenolics. In addition, the abundant components of essential oils make *Ocimum* a plant that can fight the growth of organisms. The leaves and branches were used for insecticides against mosquitoes, bees, flies, and other insects. (Pavithra, et al., 2019, Ali, et al., 2021 Kačániová, et al., 2022).



Fig. (8) Ocimum basillicum

2.10.2 Nicotiana tabacum L. (Tabaco, Fumo).

Nicotiana tabacum L., family Solanaceae (commonly known as tobacco) is one of the most economically important industrial crops worldwide and the main species for the commercial production of smokeable tobacco Approximately 6 trillion cigarettes are consumed worldwide each year and the total tobacco production is approximately 6.68 million metric tons (Hunziker, 2001).



Fig. (9) Nicotiana tabacum

2.10.3 Capsicum sp

flowering_plant of the nightshade family (Solanaceae), widely cultivated for the hot or mild peppers of its thousands of varieties and cultivars. *Capsicum annuum* is the most economically important of the species in the *Capsicum* genus. The fruits of this species are integral ingredients in the cuisines of many countries worldwide. The plant is also grown as an ornamental or as a source of medicine. Peppers can be eaten raw, cooked, pickled, roasted, or dehydrated. Pungent chilies can be used as condiments or spices for seasoning. Some peppers are also used as coloring in some foods. Pepper extracts are used in pharmaceutical products for such conditions as arthritis and athlete'sfoot. The fruits contain many phytochemicals, including vitamin C, vitamin A, flavonoids, anthocyanins, and carotenoids, as well as capsaicinoids, which are spicy components of hot peppers. The plant is an herb or small shrub that grows to a height of 0.3–1.2 meters (1–4 feet) and a width of 15–30 cm (6– 12 inches). Its glossy leaves are roughly oval with smooth margins and can reach up to 7.5 cm in length. The leaves of some cultivars turn dark purple or black as the plant grows (Juhari, et al., 2023).



Fig (10) Capsicum sp seeds

3. Materials and methods

3.1 Insect stock

Adult bruchids were obtained from infested chickpea purchased from the open market in Erbil town and were introduced into undamaged chickpea seeds maintained in large plastic container with fine net of cloth covering the opened. One male and three female weevils were introduced into the experimental samples (50g of chickpea each) for infestation, and these where subsequently studied for multiplication and grain damage daily for 30 days.

3.2 Experimental chickpea

The chickpea seeds were purchased from the open market in Erbil town sorted into undamaged clean chickpea seeds which were used for the experiment. Each sample contains 500 grains of sorted chickpea which were held in sample transparent plastic container cover with perforated clothing material.

3.3 Preparation of insecticidal plant material

The plant materials used for this experiment are tobacco leaf (Nicotiana tabacum, L.)

basil leaf (*Osmium basilicum* L.) and dry pepper powder (*Capsicum annum*, L.). All the materials used were gotten from Erbil town: tobacco leaf and dry pepper powder were purchased from local market; basil leaves were harvested from local farms. All were air-dried then ground into powdered form before usage.

3.4 Experimental procedure and design

The experiment Complete Randomized Design (CRD) consisted of twelve treatments: control, tobacco snuff (5g), basil leaf powder (5g), dry pepper powder (5g), and a combination of the treatments: 2.5g tobacco snuff + 2.5g basil leaf powder, 2.5g tobacco snuff + 2.5g dry pepper powder, basil leaf powder + 2.5g dry pepper powder, added to (300) seed, (5) pairs adult insects placed. All treatments were replicated (3) times plus control. (lledun and Chubiyojo, 2021).

3.5 Data collection and statistical analysis

Data collected include number of damaged seeds or seeds with weevil holes, number of live bruchids and number of dead bruchids. Means found to be statistically significant at 5% probability level were separated using Duncan Test.

4. Results and discussions

For individual treatments, 28 days after application of the botanicals the highest number of live weevil population was observed in cowpea stored in Basil leaf powder (7 live weevils) while the lowest live weevil population was observed when the cowpea was stored in ground Pepper (1 live weevil), which was closely followed by grains treated with Tobacco snuff, with (4 live weevils), an indication that ground pepper suppressed the most the highest number of live weevils. For all individual treatments, significant differences were observed in their outcome in response to live weevils at 7, 14, 21, and 28 days after produce were treated to the botanicals.

For the combined treatments, mixture of ground pepper powder + Tobacco snuff offered the highest control on live weevils (1 live weevil) followed by mixture of Basil leaf powder + ground pepper powder (3 live weevils), while the lowest control of the living weevils observed in the mixture of Basil leaf powder + Tobacco snuff (5 weevils).

Generally, there was significant influence of the botanicals on live weevils at 7, 14, 21 and 28 day after produce treatment with the various botanicals (Table 1) with the control treatment giving the highest number of live weevils (9 live weevils) in stored cowpea at 28 days after storage compared with those treated with botanicals.

While all the botanicals performed better than the control in regulating the number of live weevils, the best control was achieved with the use of ground pepper (1 live weevil 28 days after produce treatment) followed by mixture of Tobacco snuff + grond pepper powder (1 live weevil), then mixture of ground pepper powder + Basil leaf powder (3 live weevils). The reduction in number of live weevils is likely to translate into reduction in weevil damage, as less weevils will be available to feed on the grains, thus ensuring better produce storage. That the botanicals gave better performance compared with the control, is an indication than the botanicals have preservative abilities (Islam *et al.*, 2009).

Ability of the botanicals to induce reduction in number of weevils is in line with previous observations. Many researchers reported that plant parts, oil, extracts, and powder mixed with grain-reduced insect oviposition, egg hatchability, postembryonic development, and progeny production Asawalam and Adesiyan, 2001). Lists of 43 plant species have been reported as reproduction inhibitors against stored product insects (Talukder, 1995). Reports have also indicated that plant derivatives including the essential oils caused mortality of insect eggs Asawalam and Adesiyan, 2001). Many grounds plant parts, extracts, oils, and vapor also suppress many insects (Rajashekar *et al.*, 2010).

Previous reports have shown that plant extracts showed deleterious effect on the growth and development of insects and reduced larval pupal and adult weight significantly, lengthened the larval and pupal periods, and reduced pupal recovery and adult eclosion (Khanam*et al.*, 1990).

Treatment imposed significantly ($p \le 0.05$) negatively influenced weevil survival at 21 and 28, after the grains were treated with the botanicals (Table 2). The lowest number of dead weevils (13 dead weevils) was observed in the control treatment, while the highest number of dead weevils were in cowpea stored in ground pepper and those stored in mixture of ground pepper leaf powder + Tobacco snuff powder (25, 22 dead weevils) respectively. Formulations of the botanicals into various mixtures did not show clear patterns in respect of mixture enhancement of potency when compared with results of individual botanicals on numbers of dead weevils. For most combinations, the potency of the formulations as shown by dead weevils actually reduced relative to results of the individual botanicals.

CONCLUSION

The increasing problems of resistance and pesticide residue coupled with contamination of the biosphere associated with large-scale use of synthetic pesticides have led to calls for biodegradable pesticides. The situation is further compounded by cost of synthetic herbicides and technical-know-how required for herbicide formulation. This experiment employed plant materials: dry pepper (*Capsicum annum*), Basil leaf (*Basil* sp.) and tobacco leaf (*Nicotiana tabacum*) in the storage of cowpea against cowpea weevils. The experiment showed that while applied botanicals did not prevent weevil reproduction entirely, it enhanced weevil death compared with the control. The best result was obtained with ground pepper for the parameters investigated, however within the time limit of the experiment, complete cessation of reproduction activities among the weevils was not achieved.

Table 4: Shows the analysis of live weevils

	7 days	14 days	21 days	28 days
Control	<mark>4.00</mark>	<mark>6.00</mark>	8.00	<mark>9.00</mark>
Single applications	•			•
Pepper powder	<mark>400</mark>	<mark>2.00</mark>	<mark>1.00</mark>	<mark>1.00</mark>
Tobaccosnuff powder	<mark>2.00</mark>	<mark>1.00</mark>	<mark>3.00</mark>	<mark>4.00</mark>
Basil leaf powder	<mark>2.00</mark>	<mark>4.00</mark>	<mark>3.00</mark>	<mark>7.00</mark>
Combined treatment				
Pepper powder+Tobacco snuff powder	<mark>4.00</mark>	<mark>2.00</mark>	<mark>1.00</mark>	<mark>1.00</mark>
Pepper powder+Basil leaf powder	<mark>4.00</mark>	<mark>3.00</mark>	<mark>3.00</mark>	<mark>3.00</mark>
Basil leaf powder + Tobacco snuff powder	<mark>2.00</mark>	<mark>3.00</mark>	<mark>4.00</mark>	<mark>5.00</mark>

Table 5: Shows the analysis of dead weevils

Botanicals		Dead weevils					
		14 days	21 days	28 days			
Control	2.00	<mark>6.00</mark>	<mark>6.00</mark>	<mark>13.00</mark>			
Single applications							
Pepper powder	<mark>1.00</mark>	<mark>5.00</mark>	<mark>14.00</mark>	25.00			
Tobaccosnuff powder	<mark>3.00</mark>	<mark>8.00</mark>	<mark>13.00</mark>	<mark>19.00</mark>			
Basil leaf powder	<mark>2.00</mark>	<mark>5.00</mark>	<mark>9.00</mark>	<mark>16.00</mark>			
Combined treatment							
Pepper powder+Tobacco snuff powder	<mark>1.00</mark>	<mark>5.00</mark>	12.00	22.00			
Pepper powder+Basil leaf powder	<mark>2.00</mark>	<mark>5.00</mark>	<mark>11.00</mark>	<mark>16.00</mark>			
Basil leaf powder + Tobacco snuff powder	<mark>1.00</mark>	<mark>300</mark>	<mark>2.00</mark>	<mark>1.00</mark>			

Table 6: Shows the analysis of damaged Seeds

Rotanicals	Damaged seeds (%)						
	7 days	14 days	21 days	28 days			
Control	<mark>3.00</mark>	<mark>5.00</mark>	<mark>5.00</mark>	<mark>15.00</mark>			
Single applications	1	I	I	1			
Pepper powder	<mark>1.00</mark>	<mark>2.00</mark>	<mark>3.00</mark>	<mark>4.00</mark>			
Tobaccosnuff powder	<mark>1.00</mark>	<mark>1.00</mark>	<mark>1.00</mark>	1.00			
Basil leaf powder	<mark>3.00</mark>	<mark>5.00</mark>	<mark>5.00</mark>	<mark>8.00</mark>			

Combined treatment								
Pepper powder+Tobacco snuff powder	<mark>0.00</mark>	<mark>0.00</mark>	<mark>2.00</mark>	<mark>2.00</mark>				
Pepper powder+Basil leaf powder	2.00	<mark>3.00</mark>	<mark>3.00</mark>	<mark>4.00</mark>				
Basil leaf powder + Tobacco snuff powder	2.00	<mark>3.00</mark>	<mark>3.00</mark>	<mark>4.00</mark>				

5. References

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